Division of

Cancer Treatment

1988 Annual Report
Intramural Activities
Volume II

October 1, 1987
September 30, 1988

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

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Bethesda,
Maryland 20892

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1987 through September 30, 1988

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1987 - September 30, 1988

The Clinical Oncology Program is the intramural treatment research arm of the National Cancer Institute. The Program conducts basic and clinical research in medicine, pediatrics, surgery, pharmacology, radiobiology, endocrinology, immunology, genetics, and molecular biology in the context of developing curative therapies for cancer. The Program is composed of six Branches; Branch summaries for fiscal year 1988, with scholarly accomplishments, are listed separately. A laboratory under the supervision of Dr. Samuel Broder operates under the auspices of the Office of the Associate Director (OAD). This Office also supports a Biostatistics Data Management Section, supervised by Dr. Seth Steinberg.

PROGRAM ACCOMPLISHMENTS

OFFICE OF THE ASSOCIATE DIRECTOR

Investigator-initiated Research Activities

The OAD virtually reprogrammed all its scholarly activities in response to the AIDS epidemic and to the Department's focus on AIDS as a number one priority. A portion of Dr. Broder's investigator-initiated research activities is summarized under the following heading:

Development of Anti-retroviral Agents for the Therapy of AIDS and Its Related Disease.

AIDS and AIDS-related diseases are caused by the third known pathogenic human retrovirus, now called HIV. During the past year, Dr. Broder's laboratory has continued to develop technology for the rapid detection of drugs or biologics which can suppress the replication of HIV *in vitro*. In addition, his group has continued its efforts to bring promising drugs to clinical trials as quickly as possible. At this time, it is possible to say that the general scientific perspective on the development of anti-retroviral drugs has changed: The question no longer is *whether* clinically active drugs can be developed for the treatment of AIDS, but *how many* agents will be found and when a clinical application will be found?

A more complete discussion of various agents and therapeutic strategies will be taken up in the laboratory project report section. In summary, Dr. Broder's laboratory has focused on the development of certain anti-retroviral nucleoside analogues, their biochemical pharmacology, and their application to the therapy of patients with AIDS and related disorders.

One family of anti-retroviral nucleosides is referred to as dideoxynucleosides. The first in vitro assessment of these drugs against HIV was undertaken in this laboratory about four years ago and has been discussed in previous annual reports. About three years ago, one member of this family, AZT, was used for the first time, by the Clinical Oncology Program, to treat patients with AIDS. This drug is the first and only anti-retroviral, chemotherapeutic agent approved for prescription status.

During the past year, new studies of AZT have been undertaken. One of the primary toxicities of AZT is bone marrow suppression. In an attempt to obviate this problem, a feasibility study was initiated to test whether AZT's bone marrow suppressive activity could be ameliorated by the administration of GM-CSF. GM-CSF is a bone marrow stimulant that can promote the regeneration of cells belonging to the granulocyte and macrophage series. This is the first study of its kind in patients with AIDS. Ten patients have been studied extensively. The results suggest that GM-CSF can potentiate bone marrow function in AIDS patients and that an alternating regimen of GM-CSF and AZT is clinically active in suppressing viral replication, permitting increased T4 counts and possibly better tolerance to an AZT-related form of bone marrow suppression. While these results do not permit final conclusions, a number of larger studies will based on these results to learn whether GM-CSF can be adapted as an adjunct to anti-retroviral chemotherapy.

During the past year, the Program completed a phase I study and has continued to develop dideoxycytidine, another member of the dideoxynucleoside family. Dideoxycytidine (ddC) was shown to exert a virustatic effect in vivo in patients with AIDS and related disorders. The major dose-limiting toxicity was not bone marrow suppression (as with AZT), but rather a peripheral neuropathy. AZT does cause peripheral neuropathy. Because of these non-overlapping toxicities, a pilot study testing a regimen of weekly AZT, alternating with weekly ddC, was initiated. This regimen was found to be clinically active. Some patients have been enrolled in this regimen for more than one year without significant toxicities. The combination of AZT and ddC seems to have profoundly reduced the level of bone marrow suppression, observed with the administration of only oral AZT. The risk of peripheral neuropathy associated with single-agent ddC at high-dose continuous administration also seems to have been reduced significantly. The patients who developed a peripheral neuropathy usually had a mild, reversible form. Generally, the peripheral neuropathy did not occur during the fist six months of the regimen. These results have lead to the initiation of a large-scale, multi-center study to determine whether an alternating regimen of AZT and ddC is more active and less toxic than single-agent therapy.

In the past year, a phase I study of a third member of the dideoxynucleoside family was initiated. For the first time, the drug dideoxyadenosine was given to patients with AIDS and AIDS-related diseases. At present, this is the only study of this drug in the world. The starting dose was 0.375mg/kg, given every twelve hours. Eight patients have been enrolled in the study and third-dose escalation has been achieved. Some patients have had rises in their circulating T4 counts; other findings also suggest that this drug has activity. As yet, no dose-related toxicities have been observed. The drug was found to have a 30-40% oral bioavailability and to penetrate the central nervous system. Virtually all of the dideoxyadenosine administered to patients is rapidly converted to dideoxyinosine, a closely-related compound with anti-retroviral activity in its own right. While the results are still preliminary, and considerable caution should be exercised, dideoxyadenosine and its alter ego, dideoxyinosine, appear to be the least toxic anti-retroviral nucleosides that we have studied. In addition, if further research confirms that an every twelve-hour regimen is active, this will provide a major advantage over AZT, which currently requires an every four-hour dosing regimen.

At the beginning of the fiscal year's research effort, there was considerable uncertainty about the biology of HIV infection (particularly with respect to an important class of cells called macrophages) and the capacity of various dideoxynucleosides to affect HIV replication in such cells. Some reports suggested that macrophages provided a reservoir for HIV replication in such a way that various drugs could not affect their replication. During the past year, a rapid system for studying anti-retroviral drugs in macrophages was established. The results provided strong evidence that all of the dideoxynucleosides studied (including AZT) could block HIV replication in macrophages. However, the reasons for this were unexpected. Macrophages were found to contain low levels of kinases (activating enzymes) necessary to convert the various drugs into phosphorylated derivatives which actually suppress viral replication inside the cell. Nevertheless, in collaborative studies with Dr. David Johns of the Developmental Therapeutics Program, the normal phosphorylated nucleotides, which corresponded to the dideoxynucleoside drugs, were extremely low in macrophages compared to T cells. The low normal nucleotides meant that there was less competition for the anti-retroviral effects of the drugs against the virus at the level of its reverse transcriptase. These data may provide a better understanding for the future design of drugs as well as a more effective use of agents now at hand.

During the past year, a number of structure-activity relationships involving nucleoside analogues have bee explored. For example, an epoxy congener of cytidine has been found to possess considerable activity. This drug, 1-(2′,3′-anhydro-beta-D-lyxofuranosyl)cytosine, was prepared in collaboration with Dr. Tom Webb at Genentech, Inc. It is one of the few epoxy analogues known to possess potent anti-retroviral activity.

Although several anti-retroviral compounds are already known, almost no acyclic nucleoside derivatives (i.e., compounds which lack a sugar ring) have been reported to exert significant inhibition against HIV in vitro. During the past year, in collaboration with Dr. Jiri Zemlicka at the Michigan Cancer Foundation, a new class of acyclic compound activity against HIV-1 and HIV-2 was discovered. Adenallene and cytallene, represent two newly-synthesized compounds. A study of structural activity relationships revealed that the presence of two cumulated double bonds between the 2′ and 3′-carbons conferred anti-retroviral activity in certain pyrimidine and purine derivatives containing a four-carbon side chain. These compounds are now being evaluated for possible further development.

In the past year, a special effort has been made to identify new categories of drugs or biologics which could attack HIV at different steps in its life cycle. A great deal of attention has been focused on polyanionic polysaccharides. One representative of this class of compounds is dextran sulfate. We have observed that this agent is a potent inhibitor of HIV replication in vitro and that at least one mechanism for the observed activity is a capacity to block virion binding at the surface of target cells. Other agents which block virus binding to the cell surface are now being studied.

Finally, a major effort has been underway to deal with viral replication and expression in cells that are already infected. Virtually all the major drugs, which now are used in clinical studies, affect viral replication by protecting uninfected target cells from becoming infected by HIV. In collaboration with Dr. Jack Cohen's laboratory, certain novel oligodeoxynucleotides have been studied for capacity to inhibit viral replication. Special attention has been focused on phosphorothioate analogues, compounds in which one of the non-bridging oxygens has been replaced by a sulfur atom. Such constructs are quite stable and resistant to enzymatic attack. These compounds have a sequence-specific and non-specific activity. The sequence non-specific activity appears to be mediated by a competitive inhibition of template-primer interactions within the viral reverse transcriptase.

During the past year, we have focused on the sequence-specific inhibition. The mechanism appears to be translation arrest. We have observed that a 28-mer phosphorothioate analogue of an oligodeoxynucleotide, which is in an anti-sense configuration to the art/trs gene of HIV, can block the expression of virus even in chronically infected target cells. Full length (genomic) viral mRNA seems to undergo the greatest reduction in infected cells that are exposed to this construct. Envelope expression is affected, but to a lesser extent, while smaller transcripts such as 3'orf seem to be unaffected. This may provide the first proof that one can affect viral expression in a chronically infected target cell without necessarily killing the cell.

Thus, during the past year, with some tools at hand, the Program has tried to make an impact on AIDS and the retrovirus which causes this disease. Many of these tools were discovered or identified in Program research performed in previous fiscal years. At the same time, the Program has embarked on a campaign to find new tools for future interventions against this disease.

MEDICINE BRANCH

The major event over the past year has been the joining of the Clinical Pharmacology and Medicine Branches. The combined Branch is composed of M.D.s and Ph.D.s whose research is focused on improved diagnosis and management of patients with cancer. Significant contributions have been made in diagnostic imaging, tumor cell biology, new drug development, and clinical trial design. The following reports provide detailed documentation of these advances and focus on highlights of the past year which will dictate research in the coming year.

Diagnostic Studies

Over the past year, the Nuclear Magnetic Resonance Center (NMR) has opened and now provides an avenue to test technologies developed in our laboratories. The most noteworthy of these technologies has been observations by Dr. Cohen's laboratory that malignant transformation and multi-drug resistance may be correlated with the appearance of new signals in the phosphorus NMR spectrum. Dr. Cohen has been very active in designing surface coils capable of obtaining spectra on whole animals and humans. A major step in this process has been the demonstration by Dr. Cohen's group that signals obtained from cells in tissue culture were identical to those obtained from the same tumor cell line growing as a mass in the flank of an animal. Over the coming year, we hope to extend these observations to patients with tumor masses near the skin.

One of the major interests of the Branch has been the biochemistry and genetics of multi-drug resistance. In previous years, we have worked with tissue culture cell lines and have been able to identify a number of proteins, the presence of which correlate well with broad spectrum resistance to cancer drugs. Over the past year, Dr. Cowan's group has been able to develop monoclonal antibodies to many of these proteins. In collaboration with the Laboratory of Pathology, he is now in the process of setting up immunohistochemical tests for the expression of these proteins. We plan to test prospectively the correlation between the expression of these proteins and the clinical course of patients. A long-term goal is to use these reagents to predict prospectively the probability of response in a patient to be treated.

New Therapeutic Agents

One of the frustrating aspects of the usual process of drug development is that it is fundamentally empirical. Furthermore, the cognitive framework used does not allow the investigator is take advantage of the rapid strides being made in the molecular and cellular biology of the malignant process. We have put a high priority on the development of a framework which will utilize such information.

The best example of this is the work on anti-sense oligonucleotides. Dr. Cohen has been trying to make phosphate backbone analogues which have properties needed to make them effective drugs. The design criteria were that:

1. Any changes made must still allow hybridization to proceed.

2. The resulting structure must be able to survive in the blood stream.

3. The drug must be able to cross the cell membrane well enough to be effective.

4. The synthesis must be designed to allow pharmaceutical quantities to be made be made at reasonable cost.

A number of structures have been identified which have reasonable, but not perfect, properties. Nevertheless, some very interesting and promising observations have been made. First, the phosphorothioates appear to be very active against HIV-1 by a mechanism independent of the antisense effect, but dependent upon the high affinity these drugs have for reverse transcriptase. These agents are in preclinical development. Second, this group has identified an 80,000 dalton membrane carrier protein which appears to mediate the transport of oligonucleotides into cells. Presence of this carrier may help us get structures into cells which otherwise would not be able to enter. For this reason, Dr. Cohen will carry out extensive characterization of the specificity of this carrier. Third, antisense oligonucleotides might be used to deliver specific reagents to a target m-RNA. Major problems exist in the synthesis of such hybrid molecules becuase it is essential that there be no steric hindrance to hybridization. In addition, if the attached reagent is to contact the nucleic acid target, the three-dimensional geometry must be exact. Dr. Stein, in Dr. Cohen's lab, has overcome most of these problems and has produced antisense structures with a DNA intercalator attached. This fundamental work in organic synthesis will open the way to many novel compounds.

One of the most important events in cancer research was the identification of oncogenes. For the first time, the process of malignant transformation could be studied with the powerful techniques of molecular biology. Over the past few years, evidence on how the various oncogenes transform cells has been accumulated. One mechanism of considerable generality is based on over production or inappropriate production of various tissue growth factors. The important growth factors include TGF-a, TGF-b, PDGF, IGF-1, IGF-2, and both acidic and basic fibroblast growth factors. In addition, basic fibroblast growth factor also plays an important role in tumor angiogenesis.

We have recently shown that suramin is an active anti-cancer drug capable of eliciting responses in cancer of the adrenal gland, kidney and lymphomas. In collaboration with Dr. Lippman and Dr. Wellstein, we have shown that suramin is able to bind to and inactivate basic FGF and other tumor growth factors. As far as we are aware, this is the first drug to be identified as possibly acting through anti-growth factor mechanisms. We are now engaged in a search for analogues of suramin with better pharmacokinetic properties and less toxicity.

Another important aspect of oncogene action is through inappropriate activity of protein kinase C. This enzyme is, in turn, regulated by GTP binding proteins in the cell membrane. These G proteins represent an important link between cell surface growth factor binding sites and protein kinase C. Dr. Freter has, for the past several years, been examining the cell biology of these G proteins and their importance in malignant transformation. Over the past year, he has collaborated with Dr. Liotta of the Laboratory of Pathology to characterize a new agent which appears to selectively block tumor G protein signaling. The consequences of this are manifold and include tumor shrinkage and block of metastasis and progression. The latter is due, at least in part, to blocking the action of autocrine motility factor, a peptide important to the movement of tumor cells. The preclinical evaluation of this agent has gone smoothly. Dr. Freter is writing a protocol to test this agent clinically in collaboration with Dr. Liotta's laboratory.

Multi-drug Resistance

In previous years, we have shown that multi-drug resistance may result from the coordinate expression of a set of genes important in the detoxification of xenobiotics. Over the past year, Dr. Cowan has been able to show that one of the markers for this phenotype, glutathione transferase p, is expressed in greater than 90% of all colon tumors. This provides an excellent correlation with the resistance of this tumor to conventional chemotherapy. One of the implications of our hypothesis is that expression of this phenotype should be induced in normal tissue as a nonspecific response to injury. Dr. Cowan has been able to show that this is indeed the case for normal liver after injection of the carcinogen, 2-AAF. These findings suggest that we may be able to make normal tissues transiently drug resistant. In the coming year, we plan to test directly the hypothesis by attempting to elicit expression of this phenotype in normal bone marrow in cancer patients. If successful, we will attempt to use this to increase the dose intensity of chemotherapy.

One of the common characteristics of multi-drug resistant cells is the expression of the P-170 glycoprotein. This membrane protein mediates the ATP-dependent efflux of drugs. However, many tumor cells lack metabolically active mitochondria, the most efficient source of ATP. Thus, the possibility exists that efficiency of the P-170 glycoprotein might be limited by ATP generation. In tumor cells, the alternate to mitochondrial ATP generation is to use glycolysis, which would yield lactate as a product. To test this hypothesis, we have examined a series of drug resistant human breast cancer cell lines. There was an excellent correlation between lactate production, P-170 expression and drug resistance.

Because P-170 is so common in multi-drug resistant cells, much effort has been expended in the search for agents capable of reversing the P-170 induced drug efflux. While many agents appear to have activity in vitro, the needed concentrations often exceed those which can be obtained clinically. Dr. Fojo has shown that amiodarone is able to reverse multidrug resistance in vitro at concentrations which can be achieved in man. Based upon these findings, Dr. Fojo has designed a clinical trial to test these ideas in patients. The protocol awaits IRB approval.

AIDS Research

In addition to the work being done by people in the Branch, we actively support the clinical trials being done by Dr. Broder and Dr. Yarchoan. Within the branch, there were two areas of activity. One has been Dr. Cohen's work on phosphorothioates discussed above. The other has been the work of Dr. Allegra. Dr. Allegra has done landmark work in the treatment of the infectious complications associated with AIDS. He has shown that trimetrexate, combined with leucovorin, is very active against therapeutically intractable pneumocystis pneumonia. In addition, he has shown that it has activity against toxoplasmosis. In the coming year, Dr. Allegra plans to add dapsone to this combination.

Clinical Trials

The Clinical Pharmaology and Medicine Branches had very active clinical services with a large number of active protocols. I have chosen to review those trials currently showing promising results.

1. Breast cancer. The most interesting trial here is MB-198, a multi-modality approach to inflammatory or locally advanced non-inflammatory stage III breast cancer. This protocol uses aggressive combination chemotherapy alone with surgery and radiation therapy for local disease. The results have been outstanding, with CRs of 49% and PRs of 46%. This trial appears to be a genuine advance, especially in the treatment of inflammatory breast cancer.

The second breast cancer trial of note is the use of FU and leucovorin in previously treated patients. Of 50 evaluable patients there were 2 CRs and 10 PRs. Of the 50 patients, all but six had failed previous FU treatment. The median time to progression was 4.5 months. Because these results look so good in previously treated patients, we are planning a new front line protocol which will use FU and leucovorin in addition to cytoxan and adriamycin.

2. Lymphomas. The lymphomas remain our largest patient group and the most productive in terms of publications. It is difficult to choose what to highlight. MB-110, the randomized trial for nodular lymphomas, now has 121 patients. This study, one of its kind in several ways, is most interesting because the patients who randomized to aggressive chemotherapy have not reached

a median duration of remission at +83 months. This is radically better than previous chemotherapy results. However, the two arms are still not different in terms of survival. It seems certain that they will diverge in favor of the aggressively treated arm, but that it probably will require greater than ten years followup to do it. MB-161, the randomized trial between P-MOPP and P-Cytabom, is not out to six years and is approaching significance in favor of P-Cytobom. Were it not for the early increased incidence of Pneumocystis deaths in P-Cytobom, the results probably would be positive already. The other protocol with major implications is MB-133, a randomized comparison of radiation therapy versus chemotherapy for early stage Hodgkins disease. Conventional treatment of these patients involves radiation therapy. Our trial shows a dramatic advantage for chemotherapy, with 36% relapse rate in the radiation arm and 8% in the chemotherapy arm. Overall survival differences are now at the p=.05 level. Because the results will be so controversial, it is important to continue this trial until the results are highly significant.

NCI-NAVY MEDICAL ONCOLOGY BRANCH

Lung Cancer Clinical Treatment Trials and Molecular Biology

Chromosomal Deletion or Recessive Oncogenes:

Retinoblastoma (rb) gene abnormalities occur in small cell lung cancer and pulmonary carcinoids with 50% having 13q14 cytogenetic abnormalities and 20% exhibiting DNA abnormalities. At the mRNA level, 80% had rb abnormalities (60% showing absent rb mRNA expression and another 20% having greatly reduced levels). These studies show that the recently described rb gene plays a significant role in a common adult malignancy. (Harbour, Kaye, Gazdar and Minna)

Restriction fragment length polymorphism (RFLP) analysis has shown loss of heterozygosity for markers in the 3p and other chromosome regions in lung cancer. In addition, new cytogenetic studies show a variety of previously-unrecognized chromosomal deletions and translocations in lung cancer. These studies are leading the way to discovery of new tumor suppressor (chromosome deletion) genes in lung cancer. (Johnson, Kaye, Gazdar, and Minna in collaboration with S. Naylor, Univ. of Texas, B. Zbar, FCRC, & J. Whang-Peng, MB, CC, NIH)

Small cell-like tumors arising in extrapulmonary sites were not found to have a 3p deletion by RFLP analysis, indicating they have a different pathogenetic basis compared to small cell cancers arising in the lung. (Johnson and Gazdar in collaboration with J. Whang-Peng)

Dominantly Acting Oncogenes in Lung Cancer:

The products of the L-myc gene, first discovered in lung cancer, have been characterized as short lived, nuclear phosphoproteins, derived from several alternatively processed mRNAs, which in some cases initiate off-novel non-AUG codons. (Kaye, Birrer and Minna)

The various products of the L-myc gene have been shown to have biologic activity in transfection assays of transforming normal embryo rat cells and non-malignant rat lines to malignancy. (Birrer and Minna)

The molecular mechanisms of expression of c-myc, N-myc, and L-myc have been characterized in small cell lung cancer and have been shown to be related to differences in initiation of transcription, and loss of attenuator functions. This was the first characterization of these properties for N-myc and L-myc and the first study of this in a defined tumor type. These studies show new mechanisms of genetic changes for activation of myc family genes in lung cancer. (Krystal, Minna and Battey)

myc gene amplification in tumor cell lines and specimens was found to be more common after chemotherapy than before treatment in small cell lung cancer patients. (Johnson and Ihde)

The newly-described oncogene c-jun (related to transcription factor AP-1) was found to be expressed at very high levels in lung cancer cells of all histologic types as well as in normal lung. Because of the relationship of c-jun with tumor promotor action, this finding predicts a unique scenario for tumor promotion in the lung.

Production of Autocrine and Other Growth Factors and the Definition of Signal Transduction Pathways used by Lung Cancer Cells:

An insulin-like growth factor (most similar to IGF-1) functions as an autocrine growth factor for small cell and non-small cell lung cancer providing a new potential target for anti-tumor therapy. (Cuttitta, Mulshine and Minna in collaboration with R. Natale, Univ. Michigan)

GRP gene associated peptides (GGAPs), predicted from molecular biologic studies and demonstrated to exist by anti-peptide antibodies, have also been found to have receptors on lung cancer cells and biologic activity in stimulating the growth of lung cancer cells in vitro. These observations provide another target for anti-autocrine growth factor therapy. (Cuttitta and Minna in collaboration with R. Natale, Univ. Michigan, and John Walsh UCLA)

The presence of specific opioid receptors (mu, delta, kappa, sigma) has been found in lung cancer cells and differences between small cell and non-small cell lung cancer have been described. Of great interest, exogenously added opioids (such as morphine) will inhibit the growth of lung cancer cells in vitro with appro- priate receptor specificity. This provides a new treatment strategy based on opioids in lung cancer patients, not only to relieve pain, but also to shrink tumors. (Maneckjee and Minna)

A series of studies has defined the signal transduction pathways used particularly by autocrine growth factors, such as gastrin releasing peptide (GRP, bombesin) in lung cancer cells. These include activation of mechanisms to increase intracellular calcium and turn-over of phosphotidal inositols. The definition of these pathways leads to the design of new chemotherapies directed at interdicting signal transduction in lung cancer. In this regard, a newly-described GRP antagonist, cholera toxin, and calcium channel blockers were found to be inhibitors of small cell lung cancer growth in vitro through disrupting one or another of these pathways. Thus, agents like these are candidates for new forms of therapy. (Sausville, Treppel, Viallet and Cuttitta)

Clinical Treatment Trials and Correlations for Lung Cancer:

Clinical treatment trials in small cell and non-small cell lung cancer continue to show a great correlation between *in vitro* chemotherapy sensitivity and resistance and the response of the tumor in the patients. These results have come from a prospective randomized trial that demonstrates *in vitro* chemosensitivity testing and accurately predicts the clinical response of the patients to initial, as well as secondary, therapy. (Ihde, Johnson, Mulshine, Linnoila, Minna and Gazdar)

A detailed study of the mdr 1 gene in tumor and tumor cell lines from untreated and previously treated lung cancer of all histologic types indicates that expression of this gene is not correlated with clinical or *in vitro* chemoresistance in lung cancer. These findings direct a search for a new set of drug resistance genes in lung cancer. (Gazdar and Lai, in collaboration with Goldstein and Gottesman, DCBD)

Non-small cell lung cancer expressing neuroendocrine markers make up 12% of all cases (approximately 14,000 new cases in the USA each year) and these tumor cell lines and the tumors in patients appear relatively sensitive to currently available chemotherapy. These studies are being extended as part of a collaboration with the Lung Cancer Study Group. (Linnoila, Gazdar and Mulshine)

Screening of prospectively collected sputum cytology from heavy cigarette smokers with monoclonal antibodies, directed against small cell and non-small cell lung cancer, identifies patients with a high probability of developing clinical lung cancer. If substantiated, these findings provide for re-exploring the vital question of early detection of lung cancer. (Mulshine in collaboration with Johns Hopkins' Lung Cancer Screening Study)

Immunohistochemical detection of the surfactant associated protein of 35 kd (SAP-35) identifies a much higher than previously-recognized number of non-small cell lung cancers with bronchioalveolar properties. In addition, a panel of bronchioalveolar tumor cell lines has been developed that will prove invaluable for subsequent molecular and cell biologic studies. These findings suggest a new look at the epidemiology of lung cancer. (Gazdar and Linnoila)

Molecular Mechanisms of Peptide Hormone Gene Expression:

Significant molecular mechanisms underlying the selective expression of the human GRP gene expression in the brain has been discovered and characterized. (LeBacq and Battey)

Regulated post-translational processing of human proGRP leading to the production of a family of peptides with potential new biologic activities has been characterized. (LeBacq and Battey)

Molecular Mechanisms Underlying Malignancy and Differentiation in B Cells, T Cells, and Hematopoietic Cells:

Transcriptional regulation and attenuation of the c-myb gene has been identified in the transition from pre-B to B cells. The results are consistent with the hypothesis that marked down-regulation of c-myb is required for B cells to mature beyond the pre-B cell stage of development. (Bender and Kuehl)

The role of c-myb and L-myc expression in blocking the differentiation of hematopoietic cells has been characterized. The role of c-myc gene expression in the differentiation of teratocarcinoma cells has been characterized. (Segal)

These studies suggest that constitutive expression of normal proto-oncogenes may account for the observation that tumor cells are generally "frozen" in a particular state of differentiation. (Segal, Birrer and Kuehl)

A chromosome-14 inversion has been cloned from a T cell acute lymphoblastic lymphoma and has been shown to result in a hybrid T cell receptor gene with the immunoglobulin variable region segment and a T cell receptor constant region segment. These findings suggest that such changes predispose to malignancy and also indicate a possible receptor-mediated mechanism for this effect. (Martin and Kirsch)

A characteristic breakpoint in the T cell receptor locus has been molecularly characterized from lymphocytes of an ataxia telangiectasia patient. The results suggest that these rearrangements could predispose to malignancy in a manner analogous to Burkitt's lymphoma. (Martin and Kirsch)

DNA and RNA from lymphomas and leukemias have been characterized for immunoglobulin and T cell receptor loci to provide unique molecular "finger prints" for following tumor cell clones in these patients. These have shown distinct patterns reflecting target cell maturation for these tumors. In addition, techniques for RNA _in situ_ hybridization and direct RNA sequencing from patient samples have been developed, which will allow a molecular pathologic characterization of lymphomas and leukemias. (Felix, Seibel, Davey and Kirsch)

Major Staff and Administrative Changes in FY 1988

A series of changes in the senior staff of the NCI-Navy Branch will take place in FY 1988. These include:

Dr. Stephen Veach (CAPT, MC, USN), head of the Navy Hematology Oncology Program, is leaving to be Chief of Medicine at Oakland Naval Hospital. Dr. Veach has been a major force in the NCI-Navy Program success.

Dr. John Phares (CDR, MC, USN) will become the new head of the Navy Portion of the NCI-Navy program and continue his role as Chief of Hematology for the Naval Hospital.

Dr. Michael Anderson (CDR, MC, USN) will become the new head of Oncology for the Navy Program.

Dr. Daniel Ihde, Deputy Branch Chief, was also appointed head of the Division of Hematology/Oncology for the Uniformed Services University of the Health Sciences.

Dr. Edward Sausville will leave to become Associate Professor of Medicine at Georgetown University and the Lombardi Cancer Center.

Dr. James Battey will leave to become a Section Chief in the Institute of Neurologic Diseases.

Dr. Michael Birrer joins the senior staff as Assistant Professor of Medicine of the Uniformed Services University of the Health Sciences. He will be working on the biologic effects of oncogenes, developing transgenic mice, and developing retroviral technology for inserting new genes.

Dr. Frederick Kaye joins the senior staff as Assistant Professor of Medicine of the Uniformed Services University of the Health Sciences. He will be working on proteins regulating the expression of oncogenes and the isolation of chromosomal deletion genes.

Dr. Francine Foss joins the senior staff as Assistant Professor of Medicine of the Uniformed Services University of the Health Sciences. She will be working on the molecular biology of lymphomas and supervising the mycosis fungoides protocols.

PEDIATRIC BRANCH

A. Clinical Studies

1. Newly diagnosed patients with acute lymphoblastic leukemia:

NCI 77.02-CCG 191 - This protocol tested, in a randomized study, whether central nervous system preventive therapy using systemic high-dose methotrexate infusions alone (without cranial radiation) is equally effective and less toxic than 2400 cGy of cranial radiation and intrathecal methotrexate. 181 patients were randomized on this study. The overall remission rate was 98% with an event free survival of approximately 70% at three years for the entire study group. With a median duration on study of 76 months, there is no significant difference in the CNS relapse rate for either treatment group. Long-term follow-up evaluation of neurotoxicity (by CT scan, neuroendocrine evaluation and psychometric testing) is in progress. A recent analysis of patients longitudinally assessed with a periodic neuropsychological test battery demonstrated a striking decrease in verbal and full scale IQ in patients treated with cranial radiation and intrathecal chemotherapy. In addition, patients treated with cranial radiation and intrathecal therapy manifested significant impairment of academic achievement. No such declines were seen in the high-dose methotrexate groups. These data thus indicate that use of combined cranial radiation and intrathecal therapy can be avoided in nearly 60% of children with ALL, reducing the potential long-term neurotoxicity associated with such combined therapy. In contrast, this study has demonstrated no apparent adverse effects of high-dose methotrexate on cognitive functioning and academic achievement, confirming the value of high-dose methotrexate as central nervous system preventive therapy for children with ALL.

NCI 83P-CCG 134P - Treatment of newly diagnosed acute lymphoblastic leukemia in high-risk patients. The major aim of this study is to demonstrate that high-risk patients can be effectively treated on a regimen which uses CNS preventive therapy devoid of cranial radiation. An additional objective is to determine whether there is a difference in the outcome of patients at high risk for early treatment failure according to whether they do or do not have features consistent with the "leukemia lymphoma syndrome." The protocol involves the use of an aggressive, early intensification phase of therapy and intensive systemic maintenance therapy, together with CNS specific treatment. The latter consists of periodic administration of systemic high-dose methotrexate, systemic high-dose cytosine arabinoside and intrathecal cytosine arabinoside and methotrexate. With a median potential duration on study of 30 months, the event-free survival is projected at 83% at 12 months. The occurrence of isolated CNS relapse in only one patient to date suggests that effective central nervous system preventive therapy can be achieved without the use of cranial radiation in high-risk patients.

NCI 84A-CCG 144 - This protocol treats newly diagnosed patients in the "average-risk" category, randomizing them to one of two forms of CNS preventive therapy, either high-dose systemic methotrexate infusions or intrathecal methotrexate alone. The median potential duration on study is only 20 months. A total of 74 patients have been randomized to intrathecal methotrexate, 76 to high-dose methotrexate. There was no significant difference in either the CNS or bone marrow relapse rate in either treatment arm. At the present time the event-free survival at 12 months is approximately 90%. Additional follow-up is required before a meaningful comparison of these treatment arms can be made.

2. Intrathecal Diaziquone (AZQ) A new clinically useful agent for the treatment of meningeal neoplasia - AZQ is a lipid soluble aziridinyl benzoquinone designed for enhanced CNS penetration of the CNS to treat CNS neoplasms. Despite evidence of clinical activity demonstrated in Phase I and Phase II trials, systemic administration has been limited by severe and prolonged myelosuppression. To circumvent this problem, we evaluated the feasibility of intrathecal AZQ in a Phase I-II trial in patients with refractory meningeal malignancy. Two schedules of administration were examined: twice a week for four weeks and "CxT", every 6 hours for three doses, weekly x 4. A total of 31 patients have been treated, 23 of whom had acute lymphoblastic leukemia. Demonstrable antineoplastic activity was observed on both schedules of administration. Seven of the 16 courses delivered on the twice weekly schedule resulted in complete responses. On the "CxT" schedule, 6 of 14 courses resulted in complete responses. A maximally tolerated dose was defined for both schedules. The results of this study indicate that intrathecal AZQ has definite clinical activity in refractory meningeal malignancy, at a dose which is not associated with clinical toxicity.

3. Intrathecal 6-Mercaptopurine (6MP) - 6MP is an active antileukemic agent which has never previously been administered by the intrathecal route. Preclinical studies of intrathecal 6MP, performed in a subhuman primate model indicated that 6MP could be safely administered by the intrathecal route. Based on these studies we have initiated a Phase I study of intrathecal 6MP in children with refractory meningeal malignancy. Both a twice weekly and a concentration x time (CxT) schedule (q12h x 6 doses) are being evaluated. To date, 6 of 9 patients with ALL treated on the twice weekly schedule have responded, 4 are complete responses. There have been 4 partial responses among the 7 patients treated to date on the CxT schedule. No significant toxicity has been observed. These results indicate that intrathecal 6MP is safe and active against meningeal leukemia.

4. Thiotepa is an active alkylating agent with a steeper dose response curve than cyclophosphamide. This compound has been used with only marginal success via the intra-CSF route of administration. Our studies demonstrated, for the

first time, that substantial amounts of both thiotepa and its metabolite Tepa are present in intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent. As a result of these studies, a Phase I study of intravenous thiotepa in pediatric patients has been developed and completed. Thiotepa was administered as an IV bolus on a q 3 week schedule. The starting dose was 25mg/m^2. Nineteen patients with refractory pediatric malignancy were treated on this protocol. Dose limiting toxicity was seen at the 75 mg/m^2 dose. 65 mg/m^2 was identified as a safe dose for future Phase II trials.

5. In addition to this agent, other ongoing Phase I studies include Fazarabine, interleukin (IL-2), fludarabine phosphate and 5FU-leucovorin. We also have initiated three separate Phase II studies evaluating an intravenous 6MP approach to treat pediatric patients with brain tumors, solid tumors, and with refractory acute lymphoblastic leukemia.

6. Our primary study (77-C-145) for patients with undifferentiated lymphomas (both Burkitt's and lymphoblastic types) employs alternating cycles of a high-dose methotrexate infusion with CHOP, administered at approximately 10-day intervals without delays for neutropenia. Our analysis of 105 patients entered into this protocol permits the following conclusions: a) Complete response rate was 90% and overall long-term survival approximately 60%; b) Serum Interleukin 2 receptor levels provide the best prognostic indicator - better than stage, LDH, or even bone marrow involvement; c) The prognosis of patients with lymphoblastic lymphoma without bone marrow involvement in this protocol is excellent; d) There was no difference in outcome between patients classified as having Burkitt's versus undifferentiated non-Burkitt's lymphoma; e) Twelve patients have been entered into the new pilot protocol (85-C-62) for patients with extensive undifferentiated lymphoma. Two have had a CR and seven a PR.

Seven patients have been entered into a new pilot protocol (85-C-67) for patients with recurrent undifferentiated lymphomas (VP16, Ara-c, and Ifosfamide). Two achieved complete response and the rest partial response. This drug regimen appears to be highly active, although toxic, and we are completing the design of a new primary protocol incorporating VP16, Ara-c, and Ifosfamide at modified dosage.

7. We continue to monitor results of the completed protocol which studied the intensive treatment program for patients with high-risk pediatric sarcomas. This protocol combined high-dose chemotherapy during induction with total body irradiation (800 rads) and autologous bone marrow reconstitution. Ninety-two percent of the patients enrolled on the protocol were successfully induced. The actual disease-free survival is 50% for those patients free of metastatic disease at diagnosis versus 20% for those with metastatic disease. These results are not significantly different from historical experience and therefore do not stimulate enthusiasm for further investigating a total body irradiation, autologous bone morrow transplant approach to the treatment of these diseases.

8. Two new pilot protocols for treatment of high- and moderate-risk sarcomas are accruing patients. These protocols incorporate intensive vincristine, cyclophosphamide and adriamycin with the newly identified active combination of ifosfamide and etoposide. Twenty-seven of 30 patients on the high-risk protocol and 4 of 5 on the moderate-risk protocol are surviving free of progressive disease.

9. To continue to assess whether synergistic combinations of antibiotics are necessary for febrile granulocytopenic patients if a single antibiotic has a very broad spectrum of activity (particularly against gram negative bacteria) and achieves high-serum levels, we randomize 246 patients to either a third generation cephalosporin, ceftazidime (CTZ), or a member of the carbapenem class of antibiotics (imipenem/cilastatin), when they become febrile and granulocytopenic. To date, these agents appear comparably effective, thus offering new options for the treatment of neutropenic patients. The goal of this study is to simplify the management of cancer patients and to develop methods for treating neutropenic patients out of the hospital. In an additional study, therefore, patients are being randomized to continued antibiotic therapy with parenteral therapy (i.e., ceftazidime or imipenem/cilastatin) or a new quinolone, ciprofloxacin. To date, 63 patients have been randomized.

10. In order to prevent fever and infections, we are testing the value of passive immunization with pooled immunoglobulins (IVIG) to patients at risk for fever and neutropenia. In this double blind placebo controlled trial, pediatric and adult cancer patients are randomized to receive either IVIG or a placebo. Seventy-five patients have been randomized. No differences in the incidence or time to fever or in the incidence or outcome from infection were demonstrated.

11. Because indwelling intravenous catheters have become integral to the management of patients with cancer and because infections are the major complication associated with these catheters, we are conducting a randomized clinical trial to determine whether the incidence of catheter infection can be reduced according to the type of device being used. In this trial, patients requiring an indwelling catheter are randomized to either the standard catheter type (i.e., Hickman-Broviac) or to a subcutaneously implanted catheter (Port-A-Cath). The subcutaneous catheter requires less care and manipulation and might, therefore, be associated with lower risk of infection. To date, 100 patients have been randomized to either the Hickman or subcutaneous catheter.

12. The incidence of infants acquiring HIV infection transplacentally is increasing and the population of pediatric AIDS is expanding. With the exception of immunoglobulins, therapeutic interventions have been limited in pediatric AIDS.

To achieve plasma and cerebrospinal fluid concentrations of azidothymidine (AZT) that might provide constant inhibition of HIV replication as well as overcome the difficulties associated with administering a drug having a short half life, we evaluated the continuous intravenous delivery of AZT via a Hickman-Broviac catheter with a portable Pancreatec programmable infusion pump. Twenty-one children with symptomatic HIV disease (CDC Class P-2), ranging in age from 14 months to 12 years were enrolled. HIV was acquired by transfusion in 11 (52%), from factor replacement for hemophilia in 2 (10%), and perinatally in 8 (38%). Prior to initiation of AZT the mean CD4/CD8 ratio was 0.18 with 11 patients having CD4 counts <200/mm^3. Of note, 13/21 (62%) children entering this trial had evidence of neurodevelopmental abnormalities before beginning AZT.

Four dose levels were studied, and plasma and CSF concentrations of AZT were measured: Level 1, 0.5 mg/kg/h; Level 2, 0.9 mg/kg/h; Level 3, 1.4 mg/kg/h; Level 4, 1.8 mg/kg/h. Plasma concentrations achieved at the respective dose levels were 1.9±.3uM; 2.8±.4uM; 3.1±1.1uM; and 4.5±1.0uM. The steady state CSF:plasma ratio was 0.24±.07. Therapy was well tolerated and toxicity was limited to bone marrow suppression. Anemia (hemoglobin <8gm/dl) served as criteria for transfusion and was required in 13 patients. Dose-limiting neutropenia (<500 polymorphonuclear leukocytes/mm^3) occurred at doses 1.4mg/kg/d or above. On this basis, the recommended dosage is between 0.9 - 1.4mg/kg/d.

Dramatic improvement occurred in all 13 children presenting with encephalopathy prior to treatment. Serial IQ measurements before therapy and after 3 and 6 months of AZT showed a significant improvement ($p<0.005$) in the overall IQ score, and included improvement in both verbal IQ ($p<0.05$) and Performance IQ ($p<0.02$). Of note, 6 children without detectable evidence of encephalopathy prior to AZT also showed improvements ($p<0.01$) in IQ scores from baseline compared to 6 month follow-up, suggesting that cognitive impairment may be among the earliest manifestations of AIDS encephalopathy in children, underscoring the need for modalities that will treat the central nervous system, even in patients who do not appear to have neurodevelopmental deficits.

In addition to improvements of neurological function, patients have increases in appetite and weight, decreases in lymphadenopathy and hepatosplenomegaly, lowering of the immunoglobulin level and rises in CD4 cell number while receiving AZT. In some patients the improvements in encephalopathy occurred independent of other changes.

This study demonstrates that AZT is beneficial for children with symptomatic HIV infection, especially those who have or who are at risk for neuroencephalopathy.

In addition, we are collaborating with Duke and The University of Miami in performing an intermittent bolus infusion study with AZT. Thirty-five children have been entered into this trial of which we have enrolled sixteen patients. While improvement from neurodevelopmental delays were seen with oral intermittent therapy, the degree and consistency of improvement was significantly lower than when AZT was given by the continuous intravenous route of delivery. To further evaluate whether a steady-state of AZT is better than intermittent concentrations, a randomized trial is being initiated.

13. In addition to AZT, we are conducting a Phase I evaluation of dideoxycytidine (DDC) in children with symptomatic HIV infection. To date 5 children have been studied. Once completing the 8-week trial of DDC, children will be treated on an alternating schedule of DDC and AZT to determine whether efficacy can be maintained and toxicity lowered.

14. Disseminated neuroblastoma has a greater than 80% mortality in children greater than 1 year old at diagnosis in spite of intensive therapy including protocols with autologous bone marrow rescue. In order to explore new approaches to the treatment of neuroblastoma we have, in conjunction with Dr. Rosenberg, Surgery Branch, NCI, initiated a pilot study to treat children with recurrent neuroblastoma with interleukin-2 (IL-2) and tumor infiltrating lymphocytes (TIL). Enthusiasm for exploring this approach is based on the Surgery Branch's favorable experience with the treatment of melanoma which, like neuroblastoma, is a tumor of neural crest origin. The treatment of the first patient is in progress.

B. Pre-Clinical Studies

1. In the unique subhuman primate model for CSF pharmacokinetic studies, we have identified several agents of potential utility in treating meningeal malignancy.

We have evaluated the feasibility of intrathecal administration of 4-hydroperoxycyclophosphamide, a preactivated analog of cyclophosphamide, which does not require hepatic activation, and thus is potentially feasible for regional administration. We have evaluated the pharmacokinetics of 4-hydroperoxycyclophosphamide in the CSF following intraventricular administration. Our results indicate that therapeutic levels of this compound can be achieved in cerebrospinal fluid following intraventricular dosing. In addition we have demonstrated that intrathecal therapy with this compound is feasible and safe. Further studies of this approach are being pursued in an effort to introduce this approach into clinical studies as rapidly as possible.

Immunotoxins, antibodies linked to peptide toxins represent new potent and specific drugs potentially useful for treatment of malignancies, such as T-cell acute lymphoblastic leukemia. We have explored the feasibility of intrathecal administration of a cytotoxic anti T-cell ALL monoclonal antibody - Racin-A Chain conjugate. The pharmacology and toxicity following intraventricular administration was assessed. The CSF clearance of the conjugate appears to be by CSF bulk flow. Laboratory studies indicate that CSF concentrations of immunoconjugate exceed the LD50 for T-ALL cells by 3 1/2 logs. Following intraventricular administration, a concentration exceeding the LD50 is maintained in the CSF for greater than 24 hours. No clinical neurotoxicity was observed in these studies. The significance of a mild eosinophilic pleocytosis is being evaluated. Based on these findings, clinical studies of this approach are planned.

An innovative system which permits assessment of continuous intra-CSF drug administration has been developed in the sub-human primate. A unique lateral ventricular catheter attached to a continuous infusion portable pump, together with the 4th ventricular Ommaya reservoir enables us to deliver and monitor drug administration by continuous intra-CSF administration. Interim results with methotrexate demonstrate substantive advantages for this approach.

2. We have studied the immunoglobulin and T-cell receptor gene configuration in acute lymphoblastic leukemia of childhood. The cells of 54 patients with ALL have undergone immunophenotyping and molecular analysis. We found evidence for rearrangement of both the alleles of the gamma and beta T-cell receptor genes in the majority of cases of T-cell acute lymphoblastic leukemia. Expression of alpha mRNA was found less often, usually in the most mature T-ALL cases. Thus, analogous to studies of pre-B cell ALL, molecular analysis of T-ALL suggests a hierarchy of early gamma and beta gene rearrangement followed by subsequent alpha mRNA expression and a coordinate sequence of early appearance of the 3A1 antigen, followed by T11 and later the T3 antigen. Although this apparent hierarchy is intriguing, T-cell receptor gene rearrangement was also identified in a number of cases of pre-B cell ALL and immunoglobulin heavy chain rearrangement was occasionally seen in cases of T-cell ALL. These later cases raise significant questions regarding our preconceived concept of separate lineages of T and B cell differentiation. This information has greatly

furthered our understanding of the basic biology of the lymphoid malignancies of childhood.

3. We have also studied the effect of Interleukin-2 on immature T-cell malignancies. Immature T-cell malignancies have generally been resistant to *in vitro* differentiation by common agents such as Phorbol ester, DMSO and retinoic acid. However, we investigated the ability of recombinant IL-2 to induce phenotypic and functional differentiation in T-cell acute lymphoblastic leukemia. We were able to demonstrate that, in a significant subpopulation of cases, recombinant IL-2 induced expression of surface antigens characteristic of mature T-cells and evidence of functional differentiation in the form of acquisition of cytolytic activity characteristic of lymphokine activated killer (LAK) cells. This data suggested that IL-2 may serve to differentiate a proportion of the mature T-cell malignancies and might provide therapeutic benefit as an in vivo biological response modifier. Based on these studies, we have initiated a Phase I trial of recombinant IL-2 given by continuous infusion for pediatric patients particularly those with hematologic malignancies.

4. CNS spread of the AIDS virus is a major cause of death in patients with AIDS. Our subhuman primate model is a unique preclinical model to evaluate the penetration of systemically administered compounds into the central nervous system. A variety of antiviral compounds have been studied in this model including suramin, azidothymidine, 2′ 5′ dideoxycytidine, fluorodideoxycytidine, dideoxythymidine, azidodideoxycytidine, ribavirin, and dideoxyadenosine. Data obtained in this model is virtually identical to that observed in patients. Because of the importance of this project, a major effort is being made to study additional anti-AIDS drugs in this model.

5. Non-random chromosomal translocations, the most important being an 8;14 translocation, provide a critical element in the pathogenesis of the small non-cleaved lymphomas. We have demonstrated that chromosomal breaks occur in the first intron of the c-myc gene in some 50% of sporadic tumors, but not at all, to date, in endemic tumors. These findings strongly suggest that the mechanism of deregulation of c-myc differs in endemic and sporadic cases.

6. We have examined EBV association in the context of breakpoint location on chromosome 8 and have noted almost all far 5′ breakpoints are associated with EBV (22 of 23), including all endemic tumors examined and 3 of 4 sporadic tumors. In sporadic tumors, immediate 5′ breakpoints are rarely associated with the presence of EBV (1 of 15), although intron breakpoints are divided equally among EBV positive and negative tumors. This suggests that EBV may provide an essential pathogenetic element when the breakpoint is in some locations.

7. We have identified a series of molecular markers that define specific stages during the physiologic maturation of human fetal adrenal neuroblasts and used these markers to evaluate both cell lines and pathologic specimens of human neuroblastoma tumors. We have found that both cell lines and tumors have a pattern of gene expression that suggests they correspond to different developmental stages, although these all appear pathologically and clinically to be indistinguishable. We hypothesize that such subgroups may exist in solid tumors of other organs and that such subgroups will require individualized therapeutic regimes if the type of highly efficacious therapies currently available for some hematopoietic malignancies are to be developed for solid tumors.

8. We have developed in vitro assays to follow the maturation of human neuroblastoma tumor cell lines in culture, and we are using these in experiments to identify the molecules that regulate these maturational events. Retinoic acid, a naturally occurring morphogen, does change the developmental status of neuroblastoma tumor cells, and we are studying the molecular events by which the activity of this molecule is mediated. We anticipate that by identifying the cellular events that mediate maturation and developing classification schema to identify biologically relevant tumor subgroups it will be possible to effectively pursue the development of methods to move cells from one stage in differentiation to another.

9. We have determined that the receptor mediating the mitogenic response of neuroblastoma to Insulin-like Growth Factor II (IGF-II) is expressed in all of the twenty-five neuroblastoma tumor cell lines that we have available for study and many of these cell lines express IGF-II. Neuroblastoma tumor specimens were characterized to have a similar pattern of gene expression. In one cell line that we chose to study in detail we were able to demonstrate that the endogenously produced and secreted IGF-II functions as an autocrine growth factor, suggesting that this growth regulatory pathway contributes to the loss of growth regulation that characterizes neuroblastoma tumors.

10. We have identified a number of different genes that are specifically expressed in mature astrocytes. We are currently studying these to determine their potential as tumor markers capable of delineating subgroups of CNS tumors that correspond to cells appearing at different times during CNS ontogeny. Ultimately our goal is to develop a biologically based nosology for CNS tumors arising in astrocytes.

11. We have developed a unique model to study disseminated fungal infections in neutropenic hosts. This model closely parallels the infectious process that occurs in man and has already shed considerable light on understanding the syndrome of hepatic candidiasis and in defining appropriate antifungal regimens for use alone or in combination. We have demonstrated that the combination of amphotericin B plus 5-fluorocytosine is superior to simple agent therapy for invasive candidiasis.

We have also used this model to study the efficacy of new triazole agents, including itraconazole, fluconazole, and new Schering and Lilly azoles in models of acute lethal infection, sublethal infection, hepatic conditions and in prophylaxis. The role of indwelling catheters and their clearance (a frequent clinical problem) has also been addressed in these trials.

Studies are underway using immunoregulatory agents as adjuncts to antifungal therapy. We have studied IL-2, with and without LAK cells, both in vitro and in vivo, against fungal targets but have failed to demonstrate efficacy.

Presently, we are studying G-CSF and GM-CSF, both in vitro and in vivo, and adjuncts to our antifungal therapy. GM-CSF enhances neutrophil killing of candida.

We have studied the pharmacology of azidothymidine administered intravenously, orally and by continuous intravenous administration on ongoing Phase I trials of this compound pursued by our branch in children with HIV infection. Following IV administration over one hour, AZT disappears rapidly and in bi-exponential fashion. The values for the half-lines, clearance, and volume of distribution are similar to those reported in adults. AZT was found to be rapidly absorbent following oral administration with a bioavailability of approximately 65%. AZT levels were evaluated at 4 doses delivered by continuous infusion in 21 children. Therapeutic concentrations were sustained in plasma at all 4 dose levels. As previously shown in our primate model, the CSF/PLASMA ratio was approximately 25%; therapeutic levels of AZT can be maintained in CSF with the continuous infusion approach.

RADIATION ONCOLOGY BRANCH

The Radiation Oncology Branch continues to meet its three major goals: 1) Major emphasis on clinical trials of a combined modality nature, collaborative with other clinical branches; 2) a strong radiation biology program, with heavy emphasis on basic science, radiologic physics, and questions of clinical relevance; and 3) a training program in radiation oncology, equivalent to the stature of the programs of training in the Medical, Surgical, and Pediatric Branches within NCI.

On-going clinical work focuses on small cell carcinoma of the lung, mycosis fungoides, soft tissue sarcomas, pediatric sarcomas, lymphomas, and Hodgkin's disease. These will be presented by other respective Branches, under whose aegis the protocols are carried out.

The primary ROB studies, which have been on a moratorium for the last year, have centered around intraoperative radiation therapy. A flood in July 1986, ruining the electronics of the Microtron, has seriously limited our ability to carry out the intraoperative treatment. Also, there has been a major shift in the emphasis of the Surgery Branch because of the developments of Interleukin-II.

A primary ROB study centers on stage I and stage II breast cancer, in which patients with such carcinomas are randomized to receive either modified radical mastectomy or definitive radiation with preservation of the breast following a lumpectomy. With 225 patients randomized and despite the difficulties of such a randomization, there is no obvious superiority of either arm, suggesting that long-term results of treatment will be comparable. This study differs from the studies that Fisher of the NSABP in that the surgical excision makes no attempt to have the margins surgically negative, but simply removes the gross lump. Cosmesis is a major end-point, in addition to survival and freedom from relapse. The studies are open to women who have masses up to 5 cm, with or without nodes, thereby making breast preservation applicable to the vast majority of women in this country who present with breast cancer. All patients with positive nodes receive adjuvant chemotherapy. The psychosocial aspects are being studied.

Halogenated pyrimidines have been investigated as radiosensitizers, especially IUdR. Our first phase I study of IUdR was completed with special attention to unresectable sarcomas and gliomas. Glioma information compares favorably to other reports, with a median survival of approximately 14 months in high-grade patients, and several patients live well beyond three years. In unresectable sarcomas, we've seen striking regressions of unresectable masses, and have several patients whose masses have gone away completely with IUdR and radiation of a phase I study. The probability of local control in unresectable sarcomas has been over 60% despite the fact that these masses are generally considered radioresistant and have been typically huge in size. We have just completed an investigation to combine IUdR with small doses of FUdR to attempt to see if this could improve IUdR uptake in the tumor cells by means of blocking de novo thymidine synthesis. The upshot of this protocol was that FUdR did, indeed, improve the proportion of IUdR incorporation, but, at the same time, necessitated the reduction of the IUdR administered. In addition, the combination appeared much more toxic than IUdR alone. Thus, we have just begun to take patients with unresectable sarcomas and randomized them to receive IUdR, "yes" or "no", in conjunction with radiation therapy. We've also begun a broad protocol for IUdR in a wide variety of cancers. We hope to investigate intra-arterial IUdR for glioma, before deciding whether or not to randomize patients to intra-arterial or intravenous IUdR.

Photodynamic therapy has begun. We've utilized hematoporphyrin derivative and laser-controlled sources of light in the treatment of superficial cancers of the skin and mucous membranes. We've also used this for occluded bronchi and to peritoneal surfaces by means of intracavitary administration of the light. We hope to use the hematoporphyrin intracavitary as well, but have not yet been permitted by the FDA to do so. We also hope to use such treatment in the thorax, but again have not yet been allowed by the FDA to do so. The preliminary results on the peritoneal cases with ovarian cancer appear quite promising.

In the laboratory, the work is centered on the mechanisms of sensitization of protection, resulting from radiation modifiers, and the investigation of mechanism of action of several different cytotoxic agents. We have focused heavily on sulfhydryl compounds, especially glutathione, and its relationship to cell killing or protection by either radiation or chemotherapy. Additional work has gone on in heat shock proteins and in the characterization of human tumor cell lines in conjunction with other branches. The laboratory has demonstrated conclusively that cells, which are pleiotropically drug resistant, are not necessarily resistant to radiation therapy; moreover, such cells are definitely not resistant to photodynamic therapy. Under the direction of Dr. Gansow, we've been able to label a variety of monoclonal antibodies by means of newly-synthesized chelates to various isotopes. We have actually treated patients with radiolabelled antibody, but, at the moment, the only antibodies that have been studied in patients are the B 72.3 labelled with iodine. We hope to use other antibodies which are linked to yttrium shortly in patients.

SURGERY BRANCH

Laboratory and clinical efforts of the Surgery Branch are concentrating on the development of new diagnostic and therapeutic techniques for the management of cancer patients.

Significant laboratory accomplishments of the Surgery Branch in the last year:

1. The adoptive transfer of tumor-infiltrating lymphocytes plus IL-2 can mediate the regression of established pulmonary and hepatic metastases in a variety of murine models. These tumor-infiltrating lymphocytes are 50-100 times more potent than LAK cells in their therapeutic efficacy. In conjunction with cyclophosphamide, this treatment approach is capable of curing mice with advanced pulmonary and hepatic tumors, not susceptible to other forms of immunotherapy.

2. Significant therapeutic synergies have been seen in animal models using combined lymphokine treatment. The most effective combined treatments include the use of interleukin-2 and alpha-interferon and the use of interleukin-2 and tumor necrosis factor. These therapeutic synergies have formed the basis for clinical trials which began in the past year.

3. Using gene transfection techniques, we have demonstrated that increased expression of Class I antigens on tumors make tumors more susceptible to interleukin-2 therapy. This finding has important implications for immunotherapy because of the ability of treatment with interferon to upregulate MHC antigen expression.

4. Therapy with adoptive transfer of tumor-infiltrating lymphocytes plus interleukin-2 can be substantially enhanced by the addition of alpha-interferon. This finding has formed the basis for a clinical trial which will begin in the next year.

5. Techniques have been developed for transducing human genes into growing human tumor infiltrating lymphocytes. Genes regulating neomycin resistance have been used and work is in progress to transduce genes coding for lymphokines, such as tumor necrosis factor and alpha-interferon into tumor-infiltrating lymphocytes to increase their therapeutic potency.

6. In vitro sensitization techniques have been developed to enable the anti-tumor sensitization of lymphocytes from tumor bearing mice. These cells have been utilized in adoptive immunotherapy and provide clues for raising specific immune cells from tumor-bearing patients for use in therapy.

7. Techniques have been developed for isolating tumor-infiltrating lymphocytes from human tumors. Tumor-infiltrating lymphocytes from malignant melanomas often have specific cytolytic activity against the tumor, from which they were derived, and do not react with normal tissues or melanomas from other individuals.

8. Methods for the isolation of enriched populations of tumor infiltrating lymphocytes (TIL) from murine tumors have been developed using magnetic bead separation. Growth conditions, which result in TIL with improved in vivo efficacy against established murine tumors, have been delineated.

9. Endothelial activation occurs during IL-2 treatment with associated expression of endothelial activation antigens. This effect may be responsible for the IL-2 associated vascular leak syndrome.

10. The biology of human recombinant IL-4 has been evaluated using highly purified LAK precursors and TIL's derived from patients with melanoma. IL-4 regulates LAK precursor response to IL-2, inhibiting LAK generation prior to IL-2 exposure and enhancing it after a period of IL-2 exposure. IL-4 has also been shown to act as a T cell growth factor and enhances TIL growth with specificity from human melanoma and decreases non-specific activity from such cultures.

11. Both IL-2 and TNF administration causes the rapid emigration of the LAK precursor from the peripheral blood. The mechanism is unclear but may involve the effects of TNF on the endothelium. Further, both agents lead to the release

of detectable amounts of IL-2 in the circulation. This cytokine is readily detectable in the serum using biologic assays.

12. Extensive studies of the tolerance of normal and surgically manipulated tissues to intraoperative radiation therapy have been performed.

13. By use of the molecular technique of restriction fragment polymorphism analysis, we have identified a DNA sequence deletion in the short arm of chromosome-3 in sporadic renal cell carcinoma.

14. In a study of the effect of genitourinary tumor-produced growth factors, we have demonstrated, by Northern blot analysis, that human renal cell carcinoma has an increased expression of the transforming growth factors TGF-alpha, TCF-beta, as well as of the epidermal growth factor receptor.

15. Rat sarcomas are avid glucose utilizers, which is documented by uptake of ^{14}C-deoxyglucose and which can be used to image primary and metastatic sarcomas in rats. Similar studies in humans using ^{18}F-deoxyglucose allows accurate imaging of sarcomas by PET, and glucose uptake appears to correlate with tumor grade.

16. Leucine metabolism is altered in sarcoma patients and rates of whole body protein turnover are increased, resulting in increased synthesis and breakdown rates compared to age and sex-matched controls.

17. Tolerance to cachectin/tumor necrosis factor occurs in rats and tolerance to it provides prolonged survival to rats bearing an experimental sarcoma. Survival was not prolonged by anti-tumor effects, but rather by measurable anti-cachexia effects.

18. TNF levels increase in the serum of rats bearing sarcomas; levels correlate with tumor burden, declining food intake, and body weight. TNF levels become undetectable following tumor resection. Tolerance to TNF also protects against the lethal effects of endotoxin in rats.

19. Pineal gland hormone rapidly enhances protein synthesis in MCF-7 human breast cancer cells in mitochondria, microsomes, and cytosol.

20. Demonstrated that melatonin-induced-protein enhances RNA synthesis in MCF-7 cells and that both m-RNA and r-RNA synthesis is stimulated. Enhancement of RNA synthesis is dependent upon prior stimulation of protein synthesis by melatonin.

21. Established the in vitro sensitivity of human lung carcinoma to photodynamic therapy (PDT).

22. Established certain cellular and environmental conditions which influence the degree of cytotoxicity of PDT of human lung cancer.

Significant clinical accomplishments of the Surgery Branch include the following:

1. A new approach to immunotherapy was tested using lymphokine activated killer cells and recombinant interleukin-2 or high-dose interleukin-2 alone. In 296 patients with advanced metastatic cancer, about a 10% complete remission rate of

cancer was seen in patients with malignant melanoma and renal cell cancer, and approximately 1/3 of patients with these diagnoses undergo a 50% regression of malignancy. Attempts are underway to identify other tumor types that may be susceptible to this immunotherapeutic approach.

2. Clinical protocols utilizing tumor infiltrating lymphocytes, plus cyclophosphamide and interleukin-2 in patients, have shown that over half of patients with melanoma respond to this adoptive immunotherapy. Attempts to improve this approach to immunotherapy are in progress.

3. Clinical protocols utilizing a combination of alpha-interferon and interleukin-2 have demonstrated increased effectiveness in patients with renal cell cancer. Phase I trials have established the maximum tolerated doses of these agents.

4. Phase I trials have been completed using tumor necrosis factor and interleukin-2, and the maximum tolerated doses of these agents have been defined.

5. Phase I clinical trials using GM-colony stimulating factor have been completed, and the dose limiting toxicities have been defined. Clinical trials utilizing GM-CSF in combination with cyclophosphamide have demonstrated that the period of neutropenia can be decreased using GM-CSF.

6. Prospective randomized clinical trials of patients with soft tissue sarcomas continue to reveal an advantage to adjuvant chemotherapy in patients following surgical resection of their primary sarcoma. Current trials are evaluating the use of radiation therapy in the treatment of these patients.

7. Combinations of interleukin-2 with murine monoclonal antibodies to melanoma and colorectal carcinoma have been carried out. In the presence of radiolabeled antibodies, IL-2 has been demonstrated to prolong the whole body retention of antibody and to increase the apparent delivery to tissues. Combinations of IL-2, monoclonal antibodies at high doses and LAK cells, are now in progress.

8. Prospective randomized trials of intraoperative radiation therapy are in progress in the treatment of gastric cancer. Prospective randomized trials of patients with pancreatic cancer and retroperitoneal sarcomas have been concluded. At the present time, improved local control has been demonstrated but no improvement in disease-free or overall survival has been seen in patients receiving intraoperative radiation therapy, compared to patients receiving conventional external beam radiation therapy.

9. Techniques for the delivery of photodynamic therapy to intra-abdominal malignancies have been developed, and trials of clinical efficacy in patients with intraperitoneal carcinomatosis have been initiated.

10. The efficacy of preparative cytoreductive surgery in metastatic renal cell carcinoma patients selected for immunotherapy has been established.

11. A prospective randomized trial comparing modified radical mastectomy to excisional biopsy, irradiation, and axillary dissection has now accrued approximately 250 patients. No significant differences in disease-free or overall survival have been seen with a median followup of 4-1/2 years.

12. A prospective evaluation of 125 patients with stage I, II breast cancer treated with local excision and irradiation, demonstrate that actuarial recurrences in the breast approximate 17%. No correlation was seen between breast recurrence and degree of intraductal component, tumor size, or lymph node status.

13. A prospective evaluation of cosmetic outcome following local excision and irradiation for stage I, II breast cancer demonstrated that excellent/good results were achieved in 75% of patients.

14. A prospective study of the ability of standard techniques and intraoperative ultrasound to detect gastrinomas demonstrated that both procedures were complementary. Patients with metastatic gastrinoma have a 40% objective response rate to 5 FU, streptozotocin, and doxorubicin-combination chemotherapy. Patients with localized gastrinoma have a 43% cure rate with aggressive surgical resection of primary, occult tumors. Despite this high biochemical cure rate, gastric acid hypersecretion in patients with ZES may remain. Therefore, we warn against stopping H_2-blockers without careful study of postoperative acid output.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07251-01 CO

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phase I studies of the ddC as a single agent or with AZT

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Samuel Broder, M.D., Associate Director, Clinical Oncology Program, NCI
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Nanette Mc Atee, R.N., Nursing, Clinical Center

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In previous annual reports, the first development of anti-retroviral drugs belonging to the dideoxynucleoside family was outlined. One such drug (AZT) has now achieved prescription status. Two others, ddC and ddA, have continued in our development effort. The goal is to find drugs which, alone or in combination, have a better therapeutic index than AZT alone. In a Phase I dose-seeking trial, we administered 5 dose regimens of 2',3'dideoxycytidine (ddC), a cytidine analogue with potent *in vitro* activity against human immunodeficiency virus (HIV) which had never previously been given to man, to 20 patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC). ddC was administered intravenously for 2 weeks and then orally for 4 or more weeks. ddC was well absorbed from the gut and crossed the bloodbrain barrier in these patients. Ten of the 15 patients who received 0.03 mg/kg to 0.09 mg/kg ddC every 4 hrs had increases in their absolute number of $T4^+$ T cells at week 2 ($p<0.05$); in many of the patients, however, these rises were not sustained. Eleven of 13 evaluable patients had a fall in their serum HIV p24 antigen by week 2 of therapy ($p<0.01$); in a few patients, the p24 antigen subsequently rose to baseline while in most the decline was sustained. Dose-related toxicities included a transient symptom complex of cutaneous eruptions, fever, and mouth sores; thrombocytopenia; and neutropenia. A late toxicity appearing after 6 to 14 weeks on ddC was reversible painful peripheral neuropathy. These results suggest that ddC has activity against HIV *in vivo* and has a different toxicity profile than 3'-azido-2',3'-dideoxythymidine (AZT). Based on these different toxicity profiles, we subsequently administered a regimen of AZT (200 mg orally every 4 hrs for 7 days) alternating with ddC (0.03 mg/kg every 4 hrs for 7 days) as a feasibility study. The regimen appears active and has less toxicity than either agent alone.

INTRODUCTION

The chemotherapy of pathogenic human retroviral infections is rapidly evolving. 2',3'-dideoxycytidine (ddC), an analogue of 2'-deoxycytidine, belongs to a family of nucleoside analogues that includes 3'-azido-2',3'dideoxythymidine (AZT). These agents were shown to have activity against human immunodeficiency virus (HIV) by Mitsuya and Broder in work discussed in previous annual reports, and one agent (AZT) has been proven to induce immunologic, virologic, and clinical improvements in patients with fulmanent HIV infections. The use of AZT, however, is associated with substantial toxicity in some patients, most notably bone marrow suppression. Therefore, improved therapeutic regimens for the treatment of HIV infections are urgently needed.

Dideoxycytidine functions as a chain-terminating pyrimidine. The drug is anabolically phosphorylated (activated) in human T cells by a different pathway than the one involved in phosphorylating AZT. It is efficiently phosphorylated by human T cells and is approximately 10 times more potent than AZT on a molar basis at inhibiting HIV replication *in vitro*; complete inhibition in T cells is obtained at a concentration of 0.5 uM of ddC under conditions of high multiplicity of infection. When fewer viral particles are used, ddC is effective at even lower concentrations (down to 10 nM). The drug, like all dideoxynucleosides under discussion, is active in monocytes (see accompanying annual report). ddC is resistant to deamination by cytidine deaminase (a ubiquitous enzyme which degrades many cytidine analogues including arabinosyl cytosine). In this study, we have administered ddC to patients with HIV infection in a Phase I study designed to test the feasibility of using this agent alone or in combination with AZT in patients and to determine the dose-limiting toxicity.

MATERIALS AND METHODS

Patients

20 male patients with HIV infection, ages 25 to 57, were entered into an initial trial of ddC. Nine of the patients had AIDS with a history of *Pneumocystis carinii* pneumonia (PCP), 8 had AIDS with Kaposi's sarcoma (KS), and 3 had AIDS-related complex (ARC). An additional 6 patients with HIV infection, ages 27 to 33, were subsequently entered into a pilot trial of AZT alternating with ddC; 2 of these 6 were AIDS patients who had had PCP, and 4 had ARC. One of the 6 patients had previously participated in the initial ddC trial. Each of the ARC patients in both trials had a history of oral candidiasis.

Overall, 23 of the patients were gay, one had used intravenous drugs, and had both risk factors for AIDS. Each patient had circulating antibodies to HIV, and each had less than 350/mm^3 helper T cells (CD4$^+$ cells) when assayed prior to entry into the study. The patients were treated at the Warren G. Magnuson Clinical Center of the National Institutes of Health. The protocol was approved by the Institutional Review Board of the National Cancer Institute. Each subject gave informed consent prior to entry.

Initial trial of ddC.

ddC was synthesized for this study by the Developmental Therapeutics Program of the National Cancer Institute. Each patient received an intravenous test dose of

ddC administered over 1 hr, and some patients received an oral test dose the following day. The patients were then given ddC intravenously for 14 days according to the following regimens: 0.03 mg/kg every 8 hrs (Regimen A); 0.03 mg/kg every 4 hrs (Regimen B); 0.06 mg/kg every 4 hrs (Regimen C); 0.09 mg/kg every 4 hrs (Regimen D); or 0.25 mg/kg every 8 hrs (Regimen E). Each dose was administered over 1 hr. Patients who completed the intravenous therapy then received oral ddC at the same dose schedule for an additional 4 weeks. Patients who tolerated this initial 6 week period of therapy were given the option of continuing on oral ddC for an additional 6 to 8 weeks.

Patients were closely monitored for clinical and laboratory changes. They underwent testing for cutaneous delayed type hypersensitivity at entry and either at the end of 6 weeks or at the end of therapy (whichever came first) with 0.1 ml candida extract, intermediate strength (5 TU) purified protein derivative (PPD), tetanus toxoid, and trichophyton extract. Lymphocyte subsets reacting to Leu 3 ($T4^+$, helper-inducer T cells) or to Leu 2 ($T8^+$, suppressor-cytotoxic T cells) were analyzed by flow cytometry. Patients were also monitored for their ability to mount an *in vitro* proliferative response to tetanus, diphtheria, and A/Aichi influenza virus antigens as previously described.

Heparinized plasma samples were obtained at various times after doses of ddC for measurement of ddC levels. In some patients, an aliquot of cerebrospinal fluid (CSF) was obtained by lumbar puncture during the second week of *in vitro* therapy. ddC concentrations in the plasma samples were measured by high-performance liquid chromatography using a modification of a method previously described. ddC concentrations in the CSF samples and simultaneously obtained plasma samples were measured by mass spectroscopy as described.

Serum samples obtained at entry and weekly during therapy were assayed for HIV p24 antigen using an enzyme-linked immunosorbent assay (ELISA) developed by Abbott Laboratories (Abbott Park, Illinois). Mitogen-stimulated peripheral blood mononuclear cells were cultured for HIV as previously described at entry, at 2 weeks, and at 6 weeks.

Pilot study of AZT alternating with ddC

The AZT for this study was provided by Burroughs Wellcome Co. (Research Triangle Park, NC). After an initial evaluation, patients received a regimen of 200 mg of AZT orally every 4 hours for 7 day dosing periods, alternating with 0.03 mg/kg ddC orally every 4 hrs for 7 day dosing periods; the regimen was continued for 9 or more weeks. The patients were followed on an outpatient basis, and had weekly evaluations of their clinical, immunologic, and virologic status as described above except that viral culturing for HIV was not performed.

Statistics

The statistical significance of the rises in T4 counts and the falls in HIV p24 antigen were assessed using the one-sided Wilcoxon signed rank test for paired observations.

RESULTS

Clinical Pharmacology

The peak ddC concentration was roughly proportional to the administered dose, and peak levels of 0.5 uM (a dose providing complete protection against HIV *in vitro* under conditions of high multiplicity of infection), were attained with one-hr intravenous infusions of 0.06 mg/kg or greater. The average half-life of ddC was 1.2 hrs, and the oral bioavailability averaged 70-80%. Most of the drug appears to be eliminated by renal clearance. CSF samples obtained 2 to 3 1/2 hr after the initiation of a intravenous infusion contained an average of 20% (range 9 to 37%) of the concentration in simultaneously obtained plasma samples. Thus, ddC at least partially penetrated across the blood-brain barrier in these patients.

Immunologic and Virologic Changes

There was no clear trend in the absolute number of T4 cells in the 4 patients receiving the lowest dose schedule of ddC. However, 10 of the 15 patients receiving the next three doses (0.03 mg/kg to 0.09 mg/kg every 4 hrs) had rises in their absolute number of $T4^+$ (helper-inducer) T cells by week 2; in these 15 patients, the mean number of $T4^+$ T cells rose from $85/mm^3$ at entry to $117/mm^3$ at 2 weeks ($p<0.05$). In addition, 12 of these 15 patients had increases in their ratio of helper-inducer/suppressor-cytotoxic T cells during the first 2 weeks ($p<0.01$). After week 2, however, there were decreases in the $T4^+$ T cells in some of the patients, and by week 6 (or end of therapy if that came earlier), the mean absolute number of T4 cells ($90/mm^3$) was not significantly different from that at entry ($p>0.05$). Thus, patients receiving 0.03 to 0.09 mg/kg ddC every 4 hrs appeared to have transient increases in their T4 cells during the initial weeks of therapy.

In terms of immunologic function, 3 of the 17 patients who were anergic at entry developed positive skin tests to at least one antigen while on therapy. However, two patients (both on the 0.09 mg/kg every 4 hrs dose) who had weakly positive tests at entry lost their reactivity by week 6. The proliferative responses of peripheral blood lymphocytes to three recall antigens (influenza virus, diphtheria toxoid, and tetanus toxoid) was monitored in 15 of the patients. None had a substantial decline in their responses, and 6 had substantial improvement. In one patient (#19), there was a marked increase in the response to tetanus toxoid (3030 cpm of ^{3}H-thymidine incorporation at entry versus 24,990 cpm at week 3) which coincided with development of skin test reactivity to tetanus toxoid, and the increased proliferation may in part have been from immunization as a result of skin testing at entry. However, each of the other 5 patients with improved proliferation (#5, 9, 13, 16, and #18) responded to diphtheria toxoid (4 pts) or influenza virus (2 pts), and it is unlikely that this was the result of antigenic exposure during treatment.

There was no clear trend in the ability to isolate HIV in mitogen-stimulated cultures of peripheral blood mononuclear cells from the patients during therapy (data not shown). However, administration of ddC resulted in a decrease in serum HIV p24 antigen; of the 13 evaluable patients (i.e., those who had detectable HIV p24 antigen at some point during the study), 11 had declines during the first 2 weeks of therapy. In these 13 evaluable patients, the mean p24 antigen fell from 361 at entry to 135 pg/ml at week 2 ($p<0.01$). These decreases could not be accounted for by a change in the patients' serum antibody to p24 antigen. It is noteworthy that the falls in p24 antigen even occurred in the patients receiving the lowest dose tested. In 4 of the patients, there was a later rise in p24 antigen after these initial 2 weeks; however, at the end of the 6 weeks (or time when patients were taken off drug if earlier), the mean p24 antigen (173 pg/ml) was still less than baseline ($p<0.05$). Thus, although the patient number was small, ddC appeared to induce at least a transient fall in detectable serum p24 antigen in a significant subset of patients.

Clinical Evaluation

In 9 patients, the initial administration of ddC was stopped before the completion of the first 6 weeks. In 2 patients (#6 and #15), this was because of pneumocystis pneumonia (diagnosed during the first 2 weeks); in 6 patients, because of drug toxicity (see below); and in one patient (#1), because of high fevers (the etiology of these was unclear). The most prominent toxicity during this initial therapy was a transient symptom complex of cutaneous eruptions, malaise, fever, aphthous mouth ulcers, and to a lesser extent arthralgias, ankle edema, and/or diarrhea which were present to some degree in 14 patients, particularly those on the higher doses. Some patients had a fall in serum albumin (averaging 0.5 gm/100ml) during this period, and one patient (#14) developed lip swelling. There was considerable variation among the patients in the severity and expression of this symptom complex. It generally appeared after two weeks of therapy (range 8 days to 4 weeks), and in each of the 7 cases in which the ddC was continued after the development of the complex, the symptoms subsided after 1/2 to 3 additional weeks weeks on ddC.

Some patients receiving receiving the lower doses of ddC (up to 0.06 mg/kg every 4 hrs), developed mild transient thrombocytopenia which subsided even with continued drug administration. Three of the patients on the highest two doses developed more substantial thrombocytopenia and/or neutropenia which were dose-limiting toxicities. In contrast to patients receiving AZT in whom this is an early sign of marrow toxicity, the red blood cell mean corpuscular volume generally did not rise. Bone marrow examinations in 3 patients revealed erythroblastic vacuolization (2 patients) or no abnormalities (1 patient); megaloblastic changes were not prominant. Other toxicities, including hepatic, renal, or cardiac toxicity attributable to the drug, were not observed during this 6 week period.

A different toxicity, painful stocking-glove axonal sensorimotor peripheral neuropathy, developed in 10 of the patients who continued on ddC beyond the initial 6 weeks. It appeared to be dependent on the cumulative dose of ddC, generally appeared after 10 weeks except in the patients on the highest doses, and usually presented as a painful dysesthesia of the feet. Later, patients had decreases in light touch, temperature, vibratory, and proprioceptive sensation, and in severe cases numbness, some weakness, and absent ankle jerks. Electrophysiological studies were consistent with axonal degeneration. This neuropathy is similar to the "painful sensory neuropathy" which can develop in patients with severe AIDS, and HIV-induced neuropathy may have contributed to the picture in some patients. The neuropathy in general worsened for up to 5 weeks after the drug was stopped, but then began to gradually improve both clinically and electromyographically.

Three patients (#2, #6 and #15) developed PCP while on ddC; two of these 3 cases appeared during the first 2 weeks of therapy. Other serious infections included one case each of cerebral toxoplasmosis (patient #7), progressive multifocal leukoencephalopathy (patient #13), and fatal gram negative pneumonia (organism could not be cultured) (patient #10). This last patient was not neutropenic at the time the pneumonia developed. In total, 5 patients (#2, #3, #7, #10, and #13) have expired at the time of this writing 12 months after the initiation of the protocol; all but 1 had <30 T4 cells/mm^3 at entry.

Evaluation of clinical parameters of improvement in HIV-related symptomatology was somewhat complicated by the toxicities, particularly the fevers and the mouth sores which hampered eating. In spite of this, the patients gained an average of 0.5 kg during the first 6 weeks of the study; weight gain was particularly noticeable in the patients receiving 0.03 mg/kg every 4 hrs (mean increase 2.9 kg) and was not attributable to fluid retention. In addition, 6 of the patients reported increased energy or decreased fatigue while they were on ddC.

Pilot trial of AZT alternating with ddC

The results of this Phase I trial suggested that ddC had activity against HIV and also had a different toxicity profile than AZT. Also, because ddC appeared most active during the first 2 weeks of administration at the doses tested, we hypothesized that it might best be given on an intermittent basis; by administering ddC in an alternating schedule with AZT, we might also take advantage of their different toxicity profiles. To explore this possibility, we subsequently administered a regimen of AZT (200 mg every 4 hrs for 7 days) alternating with ddC (0.03 mg/kg every 4 hrs for 7 days) to 6 patients with AIDS or ARC for 9 or more weeks. One of the AIDS patients developed a transient episode of neutropenia, and a sepsis-like picture during week 2 and was taken off the regimen; Mycobacterium avium complex was isolated from numerous blood cultures.

The other 5 patients each completed 9 weeks of therapy. Four of the 5 patients had minimal or transient drug-related symptoms during this period and have elected to continue beyond the 9 weeks. The fifth patient had ddC-associated arthralgias during the sixth and eighth weeks on the regimen and he elected to discontinue therapy after the ninth week. One of the patients had previously participated in the phase I ddC study (#19) and he developed some burning of his feet during his first week on AZT (a late manifestation of ddC toxicity from the earlier therapy). This neuropathic pain subsequently subsided over the sub sequent 5 weeks on the AZT/ddC regimen (although it recurred in mild form after 12 weeks). The other patients have now completed up to 20 weeks of therapy without encountering neuropathy, and the first patient entered has recently had a normal nerve conduction study. Aside from the one patient mentioned above, there was no hematologic toxicity observed in the patients on this study. Overall, the patients had a mean increase of 5.4 cu micron in their red blood cell mean corpuscular volume during the first 9 weeks; this is less than one might have observed with AZT used as a single agent.

The 5 patients who completed 9 weeks on this regimen each reported an increase in appetite and had a mean 1.6 kg weight gain. Three of the patients reported increased energy and increased productivity at work. In terms of immunologic changes, each of the 5 patients had an increase in their number of $T4^+$ T cells (mean increase $48\underline{+}12 T4^+$ cells/mm^3) during the 9 weeks (from a mean of 154/mm^3 to 202/mm^3, $p<0.05$), and one of 2 patients who were anergic at entry developed skin test reactivity. Three of the patients had detectable HIV p24 antigen at entry; in each of these 3 patients, there was a decrease in antigen during the course of therapy (from 283 pg/ml to undetectable, from 174 pg/ml to undetectable, and from 408 pg/ml to 162 pg/ml). A fourth patient who had no detectable p24 antigen at entry had antigen detectable twice while on the regimen, but he was again negative by week 9. Thus, an alternating regimen of AZT and ddC appears to be tolerated for 9 or more weeks and to induce immunologic improvement and a decrease in serum HIV p24 antigen.

DISCUSSION

The results of this study demonstrate that ddC can be administered to patients with AIDS or ARC on a short-term basis; that serum drug levels above 0.5 uM (an in vitro virustatic dose) can be attained; that the drug is well absorbed when administered orally; that it has straightforward pharmacokinetics; and that it penetrates into the cerebrospinal fluid. The study revealed several toxicities associated with ddC administration: a transient cutaneous eruption symptom complex; hematologic abnormalities (which also resolved in some patients continued on drug); and, after a number of weeks, peripheral neuropathy. The results also suggest that immunologic and virologic improvement can be detected in these patients, at least transiently, as a result of ddC administration. Finally, they suggest that an alternating regimen of ddC and AZT is reasonably well tolerated for up to 18 weeks and can confer immunologic and virologic improvements in patients with AIDS or ARC.

As already discussed, ddC is a member of a family of nucleoside analogues, dideoxynucleosides, several members of which are potent inhibitors of HIV replication in vitro. Another member of this family, AZT, was shown in initial studies to induce small immunologic improvements in patients with AIDS or ARC. In a a subsequent double-blind placebo-controlled trial, AZT was shown to improve the survival of certain patients with AIDS. The T4 cell rises induced by AZT are often transient, however, and it causes substantial bone marrow suppression in many patients. A rise in the red blood cell mean corpuscular volume is an early manifestation of AZT toxicity, and it is thought that this megaloblastic marrow suppression results from AZT-induced depletion of thymidine-triphosphate levels.

Unlike AZT, ddC administration was not found to be associated with megaloblastic changes. However, as noted above, several unexpected toxicities were observed.

The mechanisms responsible for these toxicities are unknown. ddC can be more efficiently phosphorylated in certain monocyte populations than is AZT, and it can form a choline adduct in mammalian cells; it is possible that one of these biochemical characteristics might contribute to the clinical picture. Of particular interest, and encouraging in a practical sense, was that the cutaneous eruption symptom complex cleared even with continued ddC administration. The later development of peripheral neuropathy, however, ultimately limited the time that ddC could be continuously administered as a single agent using these dose schedules. (Persons taking ddC in subsequent trials should probably avoid taking other neuropathic drugs, have their vibratory sensation monitored, and have the drug stopped, at least temporarily, when early symptoms develop.) It is possible that by understanding the pathogenesis of these reactions, we might be able to prevent their occurrance while preserving the beneficial effects of ddC. The results of this study suggest that periodic drug-free intervals may significantly reduce ddC-associated neuropathy. Some patients have not been treated for more than one year without neurotoxicity.

The results of this study also showed that ddC induced at least transient (and in some patients more long-lasting) improvement in immunologic function and decreases in serum viral p24 antigen. However, ddC administration did not affect the ability to isolate HIV in mitogen stimulated cultures. This latter technique is probably not a useful indicator of an anti-HIV effect; it involves activating latently-infected cells and, unlike the serum p24 antigen, has not been shown to be affected by doses of AZT which are clinically beneficial. The decreases in p24 antigen seen in the patients were often abrupt and were observed even at the lowest dose of the drug that we tested. ddC appears to be an exceedingly potent agent, and our study provides a rationale for studying even lower doses of the drug. Indeed, preliminary results from an ongoing Phase I/II trial of ddC suggest that decreases of p24 antigen can be observed even in patients given 0.01 mg/kg every 4 hrs, and lower doses are now being tested. It is unclear why in some patients the level of p24 antigen rises after a nadir at 2 to 4 weeks (perhaps in some patients there is reduced phosphorylation of ddC after several weeks) and further research is needed to investigate this point.

The difference in toxicity profiles of ddC and AZT suggested that an alternating regimen utilizing both drugs might provide a sustained anti-retroviral effect with reduced toxicity as compared to either drug used alone; a secondary benefit of such a regimen might be that it would enable a repetition of the consistent anti-retroviral activity seen during the first weeks of ddC therapy. It appears that the hematological toxicity of AZT is drastically reduced by this approach.

We were concerned that the early ddC cutaneous symptom complex might recur during each week on ddC on such a regimen, but except in one patient, this did not occur. Indeed, the regimen tested was in general well tolerated. Also, it appeared to induce a later sustained increase in $T4^+$ T cells (comparable to that observed with AZT alone and a decline in HIV p24 antigen. It is too early to determine whether such a regimen will be better than AZT alone or indeed is substantially different than intermittent AZT administration per se (such a regimen has never been tested and might possibly provide an improved therapeutic index as compared to conventional AZT regimens); such questions will have to be addressed in larger controlled studies in which an alternating ddC/AZT regimen is compared to intermittent dosing with AZT.

In summary, the results of these trials suggest that although ddC is associated with some toxicity, it has activity against HIV in patients with AIDS or ARC even at the lowest dose tested and can be used in an alternating regimen with AZT. Additional studies will be needed to determine its role in the armamentarium against HIV infection.

Combination AZT and GM-CSF

We are also pursuing other forms of combination chemotherapy. During the past year, we have initiated a pilot study to test whether the growth-stimulating hormone GM-CSF, given in an alternating regimen with AZT, can ameliorate the bone marrow toxicity of AZT without compromising the clinical activity of AZT. The starting dose of GM-CSF was 2 mg/kg per day. After a ten-day induction regimen of GM-CSF, AZT was alternated every other week with GM-CSF.

The results of this pilot study suggest that it is feasible to give AZT (200mg q4h) in a combination regimen with GM-CSF. About two-thirds of the patients developed a localized rash at the GM-CSF injection site. This was not a serious toxicity. About an equal number had some fever during the course of therapy. The fevers were easily controlled with non-steroidal anti-inflammatory agents. Other toxicities involved myalgias and arthralgias.

While the results are still preliminary, some patients have had increases in their T4 counts and other clinical parameters on this regimen. It is too soon to draw specific conclusions; however, the regimen appears to cause less neutropenia than standard-dose AZT as a single agent.

During the next fiscal year, we will explore simultaneous administration of AZT and GM-SCF.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 07252-01 CO

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Adenallene and cytallene: Anti-HIV acyclic nucleoside analogues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Samuel Broder, M.D., Associate Director, Clinical Oncology Program, NCI

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COOPERATING UNITS *(if any)*

Dr. Jiri Zemlicka, Michigan Cancer Foundation
Dr. Shashikant Phadtaret, Michigan Cancer Foundation

LAB/BRANCH
Clinical Oncology Program, Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

While several anti-retroviral compounds are already known, very few acyclic nucleosides (derivatives lacking an oxacyclopentane) have been reported to exert significant inhibition against human immunodefiiciency virus type 1 (HIV-1) in vitro. We found two unsaturated acyclic nucleoside derivatives, adenallene [N^9-(4'-hydroxy-1',2'-butadienyl)adenine] and cytallene [N^1-(4'-hydroxy-1',2'-butadienyl)cytosine], which protect various $CD4^+$ T-cell lines from the infectivity and cytopathic effect of HIV-1. These compounds inhibit the expression of HIV-1 gag protein and suppress viral DNA synthesis at concentrations that do not affect functions of normal T cells in vitro. They also inhibit the in vitro infectivity of another human retrovirus, HIV-2. Further in vitro analyses of the anti-HIV-1 activity revealed that the presence of two cumulated double bonds between the 1' and 2' carbons and between the 2' and 3' carbons confers anti-retroviral activity in certain pyrimidine or purine derivatives containing a four carbon chain. We have also found that the 4'hydroxyl group is critical for the in vitro anti-HIV activity of adenallene. Our observations may provide new structure/activity relationships for acyclic nucleoside analogues, and may be of value in developing a new class of experimental drugs for the therapy of HIV-related diseases. The structure/activity relationships discovered so far are unexpected.

INTRODUCTION

In the relatively short time since the first clinical recognition of acquired immunodeficiency syndrome (AIDS), a great deal has been learned about the life cycle of human immunodeficiency virus (HIV) as well as the treatment of the disease. However, much remains to be learned in devising strategies for treating this disease.

We tested a number of nucleic acid base analogues which lack an oxacyclopentane moiety for *in vitro* anti-HIV-1 activity, and found that adenallene and cytallene (Suggested trivial names are based on nomenclature of nucleic bases and a suffix, allene: the adenine derivative with an allene group is designated adenallene, the cytosine derivative cytallene, the guanine derivative guanallene and the hypoxanthine derivative hypoxallene. could inhibit the infectivity, replication, and cytopathic effect of HIV-1 and HIV-2 *in vitro*. In this series of experiments we also learned about a possible structure/anti-retroviral activity relationship in such acyclic nucleoside analogues. These observations could have theoretical and clinical implications in the strategy to develop new anti-HIV drugs.

MATERIALS AND METHODS

Viruses and Cells

HIV-1 and HIV-2 were pelleted by ultracentrifugation from the culture supernatants of HTLV-III$_B$-producing H9 cells and HIV-2 producing CEM cells, and prepared to contain 5.9×10^{10} and 2.6×10^{11} virus particles per ml respectively. In the HIV cytopathic effect inhibition assay (vide infra), 0.5 and 0.005 virus particles per cell represented the minimum cytopathic doses of the virus preparations of HIV-1 and HIV-2 respectively. A CD4$^+$ Tcell clone (ATH8) and a normal CD4$^+$ tetanus toxoid-specific T-cell clone (TM11) as well as H9 cells were used as target cells for the infection by HIV in this study. Characteristics of clones ATH8 and TM11 have been described previously. Cell cultures were not synchronized as to cell-cycle.

Reagents

Adenallene [N^9-(4'-hydroxy-1',2'-butadienyl)adenine] and cytallene [N^1(4'-hydroxy -1',2'-butadienyl)cytosine] and other compounds were synthesized by Dr. Jiri Zemlicka. Detailed procedures will be published elsewhere. All tested compounds, except guanallene [N^9-(4'-hydroxy-1',2'-butadienyl)guanine], were >95% pure as shown by nuclear magnetic resonance spectra. Guanallene contained approximately 10 % of [N^9-(4'hydroxy-2'-butynyl)guanine] which is inactive against HIV-1. Adenallene, cytallene, guanallene, and hypoxallene [N^9-(4'-hydroxy-1',2'butadienyl)hypoxanthine] are racemic mixtures [50% R and 50% S form (enantiomers). 3'-Azido-2',3'-dideoxythymidine (AZT) was kindly provided by the Wellcome Research Laboratories. 2',3'-Dideoxyadenosine (ddA) and 2',3'-dideoxycytidine (ddC) were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.

HIV Cytopathic Effect Inhibition Assay

HIV cytopathic effect inhibition assays were performed as previously described. Clone TM11 cells were stimulated by 0.6 limiting flocculation units/ml of tetanus toxoid (Commonwealth of Massachusetts Department of Public Health, Jamaica Plain) and irradiated autologous peripheral blood mononuclear cells (PBM), and were cultured in complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, 15% undialyzed and heat-inactivated fetal calf serum, 50 units/ml of penicillin, and 50 ug/ml of streptomycin) containing 15% (vol/vol) interleukin 2 (IL 2, lectin-depleted; Advanced Biotechnologies Inc., Silver Spring, MD) and 25 U/ml of recombinant IL 2 (Amgen, Thousand Oaks, CA), 6 days before assay. ATH8 cells were used without the antigen stimulation. Target T-cells ($2x10^5$) were exposed to HIV for 1 hr, resuspended in 2 ml of fresh complete medium containing IL-2, and the cells incubated at 37°C in 5% CO_2-containing humidified air. Control cells were treated similarly but were not exposed to the virus. At various time points, viable cells were counted in a hemocytometer under the microscope by the trypan blue dye exclusion method.

Determination of HIV-1 gag Protein Expression

Determination of HIV-1 _gag_ protein expression was performed as previously described. Briefly, H9 cells ($2x10^4$) were exposed to HIV-1 (2,000 viral particles per cell), resuspended, and cultured at 37°C in 5% CO_2-containing humidified air. On days 6, 7, and 9, the percentage of the H9 cells expressing p24 _gag_ protein was determined by an indirect immunofluorescence by using anti-HIV-1 p24 murine monoclonal antibody (M26).

Southern Blot Hybridization

Southern blot hybridization was performed according to standard methods. Briefly, high molecular weight DNA was extracted with organic solvents, and 40 ug of such DNA was digested with endonuclease _Asp_ 718 (Boehringer, Mannheim). The digests were subjected to electrophoresis, transferred to nitrocellulose, hybridized with a radiolabelled insert of molecular clone of the _env_ region of BH10 containing a 1.3 kilobase _Bgl_ II fragment, and the viral DNA was detected by autoradiography. Relative levels of the detected viral DNA were compared by densitometry (X-Rite 301; X-Rite Inc., Grand Rapids, MI) of the exposed film.

Antigen or Mitogen-Induced T-cell Activation Assays

Washed responder TM11 cells ($1x10^5$) were cultured for 3 days with tetanus toxoid and $1x10^5$ irradiated autologous PBM in 200 ul of complete medium. In some experiments, 10^5 fresh PBM were cultured with or without phytohemagglutinin (PHA) or pokeweed mitogen (PWM) for 3 days. All cultured cells were exposed to 0.5 uCi of ^{3}H-thymidine (25.8 Ci/mmol) for the final 12 hr, harvested onto glass fibers, and the incorporated radioactivity was counted.

RESULTS

Inhibition of Replication of HIV-1 by Adenallene and Cytallene In Vitro

We tested more than 30 acyclic nucleoside derivatives which lacked an oxacyclopentane moiety for in vitro anti-HIV-1 activity in our HIV cytopathic effect inhibition assay.

We found two acyclic nucleoside derivatives, adenallene and cytallene which exerted a potent anti-HIV-1 activity in vitro. In the absence of drugs, by day 7 after exposure to HIV-1, almost all ATH8 cells were killed by the virus. However, 10 uM adenallene exerted a partial protective effect on ATH8 cells, and at 100 uM, the number of cells exposed to HIV-1 was equal to that of unexposed control cells. At higher concentrations, the drug appeared to be somewhat more toxic to the cell growth than the reference compound ddA. Cytallene at $\geq$ 0.5 uM gave a virtually complete protective effect on ATH8 cells exposed to the virus. Under the conditions used, cytallene appeared to be as effective as AZT against HIV-1 in vitro. However, it was noted that the capacity of both adenallene and cytallene to nullify the cytopathic effect of HIV-1 was often lost by day 14 of culture, while the reference compounds ddA, ddC, and AZT remained effective against the virus through the 14-day period of time (data not shown). Unlike the oxacyclopentane-containing hypoxanthine compound, 2',3'-dideoxyinosine [which is a metabolite of ddA and possesses potent activity against HIV-1 in vitro as well], the corresponding acyclic compound hypoxallene was neither effective against the virus nor toxic to the cells even at concentrations of up to 1,000 uM.

Interestingly, the corresponding guanine-containing acyclic derivative, guanallene, was not active against HIV-1 in the ATH8 system. This relative inactivity of hypoxallene or guanallene to work against the virus was confirmed both in the cytopathic effect inhibition assay as well as the HIV-1 gag protein expression using various multiplicities of infection of HIV-1 (2,000, 1,000, 200, and 100 virus particles per cell).

The protective effects of adenallene and cytallene were confirmed in different target cells, cloned normal helper/inducer TM11 cells. In the absence of the drug, HIV-1 exerted a substantial cytopathic effect on the TM11 population by day 15 in culture, resulting in a profound decrease in the number of total viable cells. However, the addition of 50 and 100 uM of adenallene or the addition of 0.5 to 2 uM of cytallene completely protected TM11 cells without affecting the growth of these normal cells.

Inhibition of HIV-1 gag Protein Expression by Adenallene and Cytallene

When the $CD4^+$ H9 cells were exposed to HIV-1 (2,000 virus particles per cells), by day 9 approximately 45% of the target H9 cells expressed p24 gag protein. In this system, adenallene at 50 uM gave a partial protective effect, and at 100 uM it completely suppressed the p24 gag protein expression by day 7; however, viral breakthrough was observed and the replication of the virus resumed in H9 cells by day 9. Using 200 uM of adenallene, however essentially no H9 cells became positive throughout the 9 days of culture. Cytallene, at 1 uM, exhibited a partial protective effect and at 5 uM, we could not detect any positive cells throughout the culture period.

Inhibition of HIV-1 DNA Synthesis in Cells Exposed to the Virus

We then asked whether viral DNA could be detected in susceptible ATH8 cells exposed to the virus but protected by acyclic nucleoside derivatives. In the absence of the drugs, viral DNA was readily detectable on day 1 and a substantial amount of the viral DNA was detected on day 3. However, in the presence of 250 uM adenallene or 5 uM cytallene, the amount of the viral DNA was markedly reduced. Densitometry readings of the exposed film showed 88.7% and 88.7% decreases on day 1, and 96.6% and 91.3% decreases on day 3 in the amount of DNA from the ATH8 cells protected by adenallene and cytallene respectively as compared to DNA from unprotected cells.

Protection of Helper/Inducer T-cells by Adenallene and Cytallene against HIV-2 Cytopathic Effects

We asked whether adenallene and cytallene could block the cytopathic effect of HIV-2. ATH8 cells were exposed to an exceedingly potent preparation of HIV-2 (20 virus particles per cell; 0.005 virus particles per cell represented the minimum cytopathic dose of the HIV-2 virus preparation).

In the absence of the drug, the HIV-2 virions exerted a substantial cytopathic effect on ATH8 cells by day 6. However, adenallene, at $\geq$ 50 uM, completely suppressed the infectivity and cytopathic effect of HIV-2. Cytallene, at $\geq$ 5 uM, also showed a complete protective effect on ATH8 cells cells against HIV-2. When this experiment was repeated, we found that the protective effect of adenallene and cytallene against HIV-2 was roughly the same as compared to that against HIV-1 under the conditions used.

Cumulated Double Bonds are Required for In Vitro Anti-HIV-1 Activity in Acyclic Nucleoside Derivatives

We explored some of the structure/antiviral activity relationships for the acyclic nucleoside derivatives described here. We first asked if the two cumulated double bonds were critical for anti-HIV-1 activity. We conducted experiments to determine whether acyclic compounds which had only one double bond in the four carbon chain were effective against HIV-1 *in vitro*. All such compounds tested failed to protect ATH8 cells against the cytopathic effect of HIV-1 at the concentrations tested. Compounds which had one triple bond in the four carbon chain were not effective against the virus. All other acyclic compounds without the two-double-bond conformation failed to suppress the virus.

We then asked if the hydroxyl group at the 4'-carbon was necessary for the anti-HIV-1 activity of acyclic nucleosides. To test this, we substituted the hydroxyl group of adenallene with a chlorine and obtained 4'-chloro-4'deoxy-adenallene. Interestingly, this substitution nullified the capacity of adenallene to block the replication of HIV-1, indicating that the 4'-OH group is required for the antiviral activity.

Effect of Adenallene and Cytallene on the Antigen- or Mitogen-Induced Activation

Finally, we tested the effects of adenallene and cytallene on the in vitro immune reactions of TM11 cells and PBM from normal individuals. Adenallene showed no inhibitory effect on the antigen-induced proliferation nor did it inhibit PWM- and PHA- induced T-cell activations at concentrations up to 50-100 uM. The proliferation of both antigen-activated normal TM11 cells and PWM-activated PBM was unaffected by cytallene at concentrations up to 10 uM, while that of PHA-activated PBM was moderately suppressed by 10 uM cytallene.

DISCUSSION

In the current study, both adenallene and cytallene showed a potent activity against HIV-1 and HIV-2 in vitro; however, both in the ATH8 cell cytopathic effect inhibition assay and in the H9 cell HIV-1 gag protein expression assay, some viral breakthrough was observed. Furthermore, when we looked for the presence of HIV-1 proviral DNA, certain amounts of viral genome were detected in ATH8 cells protected by these compounds. We and others have previously observed a similar resumption of HIV-1 replication or escape of the virus in certain cell systems in the presence of AZT. The antiviral effects of such drugs depend not only on an adequate concentration of the nucleosides, but on adequate levels of certain kinases that are cell-cycle dependent. The apparent resumption of viral replication in the presence of adenallene and cytallene might perhaps be due to catabolism of these compounds or to infection of certain cell populations (which then in turn produce more virus for variable periods) having an ineffective capacity to activate the drugs at a given point of time. The precise way in which HIV-1 resumes replication in certain culture systems in the presence of these drugs is not established and will require further research.

Unlike 2',3'-dideoxyinosine, which can give rise to 2',3'-dideoxyadenosine 5'-triphosphate inside human cells and exerts a potent anti-HIV effect in vitro, hypoxallene was neither effective against the virus nor toxic to the cells even at 1 mM. We also found that guanallene was not active against inhibitors for target viral enzyme(s) (e.g. reverse transcriptase) in spite of intracellular activation. These issues might be resolved in further studies of the biochemical pharmacology of these drugs.

We have recently obtained data that both adenallene and cytallene are stable at low pH. These drugs are resistant to pH 1 at room temperature and do not degrade for at least 16 hours. Such data might make them potentially suitable for regimens that involve prolonged therapy, since from our experience with other nucleosides including AZT and ddC, it is possible that adenallene and cytallene will be absorbed by oral administration. Taken together, our observation may be of value in developing a new class of experimental drugs for the therapy of HIV-related diseases.

PUBLICATION

1. Hiyashi S, Phadtare S, Zemlicka J, Matsukura M, Mitsuya H, Broder S. Adenallene and cytallene: acyclic nucleoside analogues that inhibit replication and cytopathic effect of human immunodeficiency virus in vitro. Proc Natl Acad Sci, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07253-01 CO

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Dideoxynucleoside activity against HIV in monocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of the probable role of HIV-infected monocyte/macrophages in the pathogenesis and progression of AIDS, it is essential that antiretroviral therapy address viral replication in cells of this lineage. Several dideoxynucleosides have been shown to have potent in vitro and, in the case of 3'-azido-2'3'-dideoxythymidine (AZT) and 2'3'-dideoxycytidine (ddC), in vivo activity against HIV. However, because these compounds must be phosphorylated (activated) in target cells and because monocyte/macrophages may have levels of kinases that differ from those in lymphocytes, we investigated the capacity of these drugs to suppress HIV replication in monocyte/macrophages using HIV-1/HTLV-III_{Ba-L} (a monocytotropic isolate). In the present study, we observed that HTLV-III_{Ba-L} replication in fresh human peripheral blood monocyte/macrophages was suppressed by each of 3 dideoxynucleosides: 3'-azido-2'3'-dideoxythymidine (AZT), 2'3'-dideoxycytidine (ddC), and 2'3'dideoxyadenosine (ddA). Similar results were observed in 5 day-cultured monocyte/ macrophages, although higher concentrations of the drugs were required. We then studied the metabolism of AZT and ddC in such cells. The phosphorylation of ddC to a triphosphate moiety was somewhat decreased in monocyte/macrophages as compared to H9 T-cells. On the other hand, the phosphorylation of AZT in monocyte/ macrophages was markedly decreased to 25% or less of the level in T-cells. However, when we examined the level of the normal endogenous 2'-deoxynucleoside triphosphate pools, which compete with 2',3'-dideoxynucleoside triphosphate for viral reverse transcriptase, we found that the level of 2'-deoxycytidine-triphosphate (dCTP) was 6-8 fold reduced and that of 2'-deoxythymidine-triphosphate (dTTP) was only a small fraction of that found in T-cell lines. These results suggest that the ratio of dideoxynucleoside-triphosphate to normal deoxynucleoside-triphosphate is a crucial factor in determining anti-viral activity of nucleoside analogues in monocytes.

INTRODUCTION

The infection and destruction of T-lymphocytes by human immunodeficiency virus (HIV) has been considered a central event in the pathogenesis of the acquired immunodeficiency syndrome (AIDS). There is a growing body of evidence, however, that infection of cells in the monocyte/macrophage series by HIV may be clinically as important as that of T-cells. Certain strains of HIV can replicate in monocyte/macrophages for a considerable time (>6 months) without necessarily inducing a substantial cytopathic effect. Such cells may thus serve as a reservoir for dissemination of the virus to other cell types. Furthermore, HIV infection can perturb accessory cell function without killing cells (Mitsuya, H. and Broder, S., unpublished data). Finally, monocyte/macrophages are believed to be important target cells in the pathogenesis of AIDS-related dementias.

Our group has recently reported that several members of a family of compounds known as dideoxynucleosides (ddN) are potent inhibitors of HIV replication in human T-cells in vitro, and at least two such compounds, 3'-azido-2'3'-dideoxythymidine (AZT) and 2'3'-dideoxycytidine (ddC) have been shown to have antiretroviral activity in patients with AIDS and AIDS-related complex (ARC). Several of these drugs are currently being studied in clinical trials, and these topics are covered in other sections of this annual report.

Dideoxynucleosides are thought to require anabolic phosphorylation to an active triphosphate moiety by kinases in the relevant target cells. As triphosphates, these compounds act at the level of the viral reverse transcriptase, competing with the normal nucleoside triphosphates and possibly terminating proviral DNA chain elongation. These drugs can prevent uninfected cells from becoming so, but they do not eliminate virus from chronically infected cells.

It is known that monocytes and macrophages may have lower levels of nucleoside kinase activities compared to replicating T-cells, although the absolute levels of these enzymes may be affected by the status of differentiation of monocyte/macrophages. It has also been reported that certain members of the dideoxynucleoside family may be phosphorylated more slowly in monocyte/macrophages than in T-cells. Because of these differences and the importance of monocyte/macrophages in HIV infection, we examined the ability of three dideoxynucleosides that are currently being evaluated in clinical trials to inhibit HIV replication in monocyte/macrophages (M/M), and correlated the activity of two of them with the concentration of their triphosphorylated moiety in such cells.

In this study we have learned that each of these dideoxynucleosides tested can suppress the in vitro replication of a monocytotropic strain of HIV, HTLV-III$_{Ba-L}$, in monocyte/macrophages, at concentrations comparable to those at which T-cells are protected. We further show that although the phosphorylation of at least one of these compounds, AZT, is substantially reduced in M/M as compared to T-cells, there is also a parallel reduction in the levels of the competing normal deoxynucleoside triphosphate. Taken together, the results suggest that the ratio of the dideoxynucleotide-triphosphate (ddNTP) to the normal deoxynucleotide-triphosphate (dNTP) may be a crucial factor in determining the activity of dideoxynucleosides against HIV.

MATERIALS AND METHODS

Drugs

3'-azido-2'3'-dideoxythymidine (azidothymidine or AZT) (Wellcome Research Laboratories, Research Triangle Park, NC), 2'3-dideoxycytidine (ddC), and 2'3'-dideoxyadenosine (ddA) (Pharmacia Inc, Piscataway, NY) were diluted in sterile distilled water or in RPMI 1640 and kept at 4°C until used. ^{3}H-ddC (specific activity: 27 Ci/mmol) (Research Triangle Institute, Research Triangle Park, NC) and ^{3}H-AZT (specific activity: 3 Ci/mmol) (Moravek Biochemical, Brea, CA) were stored dried and used immediately after reconstitution.

Virus

Two different strains of HIV-1, HTLV-III$_{Ba-L/85}$ and HTLV-III$_{B/83}$, were used in these experiments. HTLV-III$_{Ba-L}$, which was originally obtained from a sample of lung tissue and replicates efficiently in monocytes, was maintained in cultures of normal peripheral blood monocytes. HTLV-III$_{B}$, which efficiently replicates in cultured T-cells, was obtained from the concentrated supernatant of infected H9 cells (Electro-Nucleonics Lab., Inc. Silver Spring, MD). Different strains of HIV-1 were used to infect T-cells and M/M populations because we obtained only inconsistent infection of M/M using HTLV-III$_{B}$, and similarly inconsistent infection of immortalized T-cell lines using HTLV-III$_{Ba-L}$. The viral preparations were stored in liquid nitrogen. All viral preparations were tested and found to be free of contamination with mycoplasma.

Cells

Monocyte-enriched populations of peripheral blood leukocytes were obtained from healthy, HIV-negative donors, using a Fenwal C3000 cell separator. These cells were further enriched for monocyte-macrophages by centrifugation over Ficoll-Hypaque (Organon Teknika Cor., Durham, NC.). An additional purification of monocyte/macrophages (M/M) was done utilizing three different methods. 1) fresh M/M were obtained by elutriation using a Beckman J-6B centrifuge (Beckman Instruments, Palo Alto, CA). 2) Fresh M/M were also obtained by two hour-adherence to 75 cm^2-plastic flasks (Costar, Cambridge, MA.), in RPMI 1640 medium (Gibco Lab., Grand Island, NY) supplemented with 20% heat-inactivated fetal calf serum (FCS) (Gibco Lab., Chagrin Falls, OH), 2mM L-glutamine, 50 U/ml penicillin and 50 ug/ml streptomycin (Gibco Lab., Grand Island, NY), referred to as "complete medium". The non-adherent cells were then removed by three washing with warm RPMI 1640 medium and the adherent cells detached by a 15 min. incubation at 37°C with 10 ml of 0.53 mM Versene 1:5000 (Gibco Lab., Grand Island, NY.) at 37°C. 3) Five day-old adherent cells were obtained by incubating 10^6 peripheral blood mononuclear cells (PBM)/well in 48 well(1 cm^2-well) plates (Costar) in 1 ml of complete medium. Non-adherent cells were removed by washing the wells several times with RPMI 1640 at 37°C, and the adherent cells were left attached. In general 0.8x10^5 to 1.5x10^5 viable cells remained attached to each well as detected by trypan blue exclusion.

Viability of the cells obtained by each of these techniques was always greater than 95% as determined by trypan blue exclusion. In each population, 95% or more of the cells had the morphologic characteristics of monocyte/macrophages when examined after Giemsa staining (Gibco Lab., Grand Island, NY). Five day-adherent cells were consistently >97% positive for non-specific esterase (Technicon Instruments Co., Tarrytown, NY), while the elutriated and 2 hr-adherent cells were >80% esterase positive when initially prepared; these latter two preparations became >95% esterase positive after being cultured for 5 days in complete medium. The 5 day-adherent cells were >75% positive for OKM1 (Ortho Diagnostic System, Inc., Raritan, NJ.), while the elutriated and 2 hr-adherent cells were consistently >95% positive for OKM1. Cells prepared by each of the techniques were <1% E-rosette positive and <1% OKT11-positive (Ortho). Two cell lines were used in certain experiments: ATH8, an HTLV-1-infected T4-positive cell clone which is highly sensitive to the cytopathic effect of the HIV-1 (HTLV-III$_B$ strain), and H9, a T4-positive cell line; these lines were both mycoplasma free. In some experiments, macrophage-depleted peripheral blood lymphocytes were used; they were consistently <2% positive by non-specific esterase staining.

Assays of antiviral activity. Immediately after separation, 10^6 fresh M/M (prepared by either elutriation or 2 hour-adherence) were seeded in 24 well-plates (2 cm^2/well) (Costar) in 2 ml of complete medium. In some experiments, 1 cm^2- or 4 cm^2-well plates were used, keeping the same cell concentration: no difference was seen in the results. Also, in a few experiments, 10^5 fresh elutriated M/M were cultivated in 1 ml of complete medium in 1 cm^2-well plates for 5 days prior to drug exposure and challenge with HTLV-III$_{Ba-L}$. Five-day adherent M/M were allowed to remain attached in the 1 cm^2-wells in 1 ml of complete medium after the non-adherent cells were washed off on day 5. Neither interleukin-2 (IL-2) nor mitogens were added to any of the monocyte/macrophage cultures. In preliminary experiments, we found that preincubation of M/M with polybrene did not improve infection of the cells; thus polybrene was omitted in these experiments.

Prior to exposure to HIV, the target cells were preexposed to various concentrations of AZT, ddC, or ddA for 20 min.; they were then exposed to HIV without washing out the drugs. Unless stated otherwise, 80,000 cpm/ml reverse transcriptase (RT) of HTLV-III$_{Ba-L}$ were used in the experiments; this represented 0.16 RT cpm/cell for the elutriated and 2-hour adherent cells and 0.5-1 RT cpm/cell for the 5 day-adherent cells (depending on the number of cells remaining attached to the wells). This dose of virus generally yielded the maximum infection of the M/M populations, and was 16-100 times the minimum infective dose (depending on the number of cells present in the wells). In some experiments, 3,000 viral particles/cell of HTLV-III$_B$ were used to infect ATH8 or H9 T-cell lines; this is 60 times the minimum infective dose for ATH8 cells. Appropriate positive and mock-infected negative controls were run for each experiment.

After infection, the cells were maintained at 37°C in a humidified atmosphere supplemented with 5% CO_2. Two days after infection, M/M were extensively washed in order to remove excess virus, and cultivated in 2 ml of complete medium with the same concentration of drugs as before. Cells were washed and fed every 4 days.

In order to evaluate any initial virus remaining after washing, in each experiment 1 ml of complete medium plus 80,000 RT cpm/ml of HTLV-III$_{Ba-L}$ was added to each of 2 wells in the absence of cells. The wells were washed and fed fed at day 2, and subsequently every 4 days, comparable to the procedure for cell-containing wells. The initially added virus was considered absent when both wells contained undetectable HIV-p24 antigen.

Viral detection: Starting from day 3, HIV production was assessed at regular time points until day 28 using 2 different methods: measurement of released RT activity, and detection of HIV-p24 antigen production in culture supernatant. To measure RT, 1 ml of cell-free supernatant was precipitated with 0.5 ml polyethylene-glycol (M.W. 8000) (Sigma Chemical Co, S.Louis, MO), and the RT was assayed using a minor modification of a technique previously described. Briefly, 10 ul of protein extract was incubated for 1 hour at 37^{o}C with 50 mM tris-HCl, pH 7.8, 10mM $MgCl_2$, 10mM DTT, 20uM ^{3}H-dTTP (Dupont-NEN Product, Boston, MA) and 5 ug of poly(rA)-oligo$(dT)_{12-18}$ (Pharmacia Inc., Piscataway, New York) in a final volume of 100 ul. The reaction was stopped with 10 ug of yeast transfer RNA plus 1 ml of chilled 10% trichloroacetic acid containing 0.02% sodium pyrophosphate. The precipitates were collected on glass fiber filters, washed and dried; after addition of 10 ml of non-aqueous liquid scintillation cocktail (Du Pont-NEN Products, Boston, MA), the radioactivity was determined with a Beckman Model scintillation spectrometer. HIV-p24 antigen was assessed by a sandwich enzyme-linked immunosorbent assay (ELISA) (Dupont Co, Wilmington, DE). With these 2 methods, a small amount of residual virus (50-100 pg/ml of p24) was sometimes present at day 3. However, residual virus was never detected at day 7 or later. Additional experiments were performed using unstimulated peripheral blood lymphocytes, H9 cells and ATH8 cells. H9 cells were cultivated in complete medium without IL-2. ATH8 cells were cultured in complete medium supplemented with 15% human natural IL-2 from ABI, Silver Spring, MD, and 20 U/ml human recombinant IL-2 from AMGen Biological, Thousand Oaks, CA. In the experiment with ATH8 as a target of HIV-infection, protection from the cytopathic effect of HIV/HTLV-III_B (a measure of antiretroviral effect) was assessed by trypan blue exclusion. It is worth stressing, however, that one must be cautious in comparing the activity of drugs in the T-cell and M/M assay systems used in this work, because different strains of HIV were, of necessity, used in the two cell types. Drug toxicity was evaluated at various times by trypan blue exclusion in mock-infected, drug-exposed M/M.

Metabolism of dideoxynucleosides

5 x 10^6 fresh elutriated M/M and 2 hour-adherent M/M (immediately after separation), 5 day-old M/M (immediately after final purification), and H9 cells were plated in 2 ml of complete medium in 2 cm^2-well (Costar), and treated for 24 hours with 0.5uM undiluted, ^{3}H-radiolabeled ddC, or with 10uM undiluted, ^{3}H-radiolabeled AZT. The cells were then harvested (M/M by gentle scraping followed by washing with cold RPMI), counted (viability was always >92%), and washed three times in cold RPMI in order to remove excess radioactivity, pelleted and immediately frozen in dry ice. Cell pellets were then extracted with 0.5ml 10% trichloracetic acid; the acid extract was neutralized with an equal volume of tri-n-octylamine in freon, after which the neutralized extracts were analyzed via high pressure liquid chromatography (HPLC) for metabolites. Results were given as pmole of ddCTP or AZTTP /10^6 cells.

Deoxyribonucleoside-5'-triphosphate pools: $5x10^7$ elutriated, 2 hour-adherent, or 5 day-adherent M/M were collected immediately after preparation, washed at 4°C with RPMI, pelleted and stored at -70°C; a similar procedure was followed for H9 cells. Endogenous deoxyribonucleoside-5'-triphosphate pools (dNTP) were analyzed by anion exchange HPLC: pellets were extracted with 10% trichloracetic acid and neutralized as shown in the previous paragraph. Ribonucleotides were degraded using a modification of the periodate procedure of Garrett and Santi. The HPLC system utilized a Whatman Partisphere 5u SAX column (Whatman International Limited, Maidstone, England) and a gradient of 0.01 M to 0.6 M ammonium phosphate, pH 3.5.

Nucleoside kinase assay: 10^7 fresh elutriated M/M, 5-day-old M/M, and H9 cells prepared as described above were washed in cold RPMI; the pellet was resuspended in 200 ul of 2 mM DTT and stored at -70°C. 2'-Deoxycytidine-kinase (dC-kinase) and 2'-Deoxythymidine kinase (dT-kinase) activities were measured by a minor modification of a published procedure using 10 or 2000 uM 2'-deoxycytidine or 430 uM 2'-deoxythymidine as substrate in the presence or absence of 500 uM ATP-Mg.

RESULTS

Time course of infection of monocyte/macrophages by HTLV-III_{Ba-L}

The time course of HIV production by fresh elutriated- and 5 day-adherent M/M following exposure to HTLV-III_{Ba-L} was established. No detectable viral production was found during the first 3 days after viral exposure. However, starting at about 10 days, high-titer viral production was obtained which was sustained for at least 4 weeks. In most experiments we observed greater virus production in 5 day-adherent M/M than in fresh (elutriated or 2 hour-adherent) cells.

The efficiency of infection was dependent on the concentration of the added virus. Peak infection was obtained with 80,000 RT cpm/ml of culture. When higher concentrations of virus were added to the cultures, the viral production was often lower, possibly because of early interference by incomplete viral particles. It is also conceivable that a cytopathic effect of the viral preparation may have contributed to this; however, little or no cell death was observed when HTLV-III_{Ba-L}-exposed M/M were assessed by trypan blue exclusion.

Little or no viral production was found in cultures of monocyte-depleted peripheral blood lymphocytes exposed to HTLV-III_{Ba-L} in the absence of PHA and IL-2.

Anti-HIV activity of dideoxynucleosides in M/M

Based on these initial experiments, we examined the anti-HIV effect of several ddN in cultures of M/M exposed to 80,000 RT cpm/ml of HTLV-III_{Ba-L}. In cultures of fresh M/M (obtained either by elutriation or by 2 hour-adherence), we found $\geq$95% suppression of HIV replication with concentrations of 0.05uM AZT, 0.01uM ddC, or 0.05uM ddA. These concentrations of drugs are lower than those required to suppress the virus in H9, a replicating T-cell line, against infection with the T-cell tropic virus HIV/HTLV-III_B. Protection was sustained throughout the

28 day-culture period. In additional experiments, no viral production was detected when either fresh or 5-day-adherent M/M were exposed up to 320,000 cpm/ml RT of HTLV-III_{Ba-L} in the continuous presence of 0.5uM ddC.

Each of the ddN tested could also suppress HTLV-III_{Ba-L} replication in 5 day-adherent M/M, although in this cell population somewhat higher concentrations of drugs were required. In most experiments, we observed >95% inhibition of viral replication in 5-day-old M/M with 0.1uM AZT, 0.1uM ddC, or 1uM ddA. Similar results were obtained when less virus (20,000 cpm RT/ml) was used to infect the cells. Again, viral inhibition was sustained for each drug during the whole 28-day period assayed. In additional experiments, we examined the effect of dideoxynucleosides on HIV infection of fresh elutriated M/M which were allowed to mature for 5 days in culture prior to their being exposed to virus. AZT was slightly less potent in this cell population than either fresh or 5-day adherent cells; the inhibitory dose 50% (ID_{50}) was approxymately 0.1 uM. The activities of ddC and ddA were roughly similar in these cells to their activity in 5-day adherent cells (ID_{50} approximately 0.001 uM for ddC and 0.1-1 uM for ddA).

In some experiments, elutriated or 5 day-old M/M were subjected to 3 cycles of freezing and thawing at day 21, with the aim of releasing viral antigens which might be in the macrophages; this technique has been shown to be effective at releasing viral antigens in other cell culture systems and has been recently reported by Gendelman et al. to induce the release of reverse transcriptase from HIV-infected macrophages cultured with colony stimulating factor. No additional p24 antigen was detected in the supernatants following this procedure. Also, in 3 different experiments, HIV production was assayed by a determination of RT activity in culture supernatants; these results were essentially parallel to those assessed by p24 antigen production. Finally, M/M exposed to high multiplicity of infection of HIV-1 in the form of HTLV-III_B (a viral isolate which can readily replicate within T-cells) showed inconstant low-level infection; however, that viral production which did occur was consistently inhibited by ddN.

Consistent with the monocytotropic nature of HTLV-III_{Ba-L}, ATH8 cells (an immortalized T-cell clone) exposed to HTLV-III_{Ba-L} at 80,000 RT cpm/ml failed to produce detectable virus up to day 14 after viral exposure, while only transient and low-level viral production was observed in H9 T-cells exposed to this strain. Using HTLV-III_B, >95% protection at day 14 was obtained in both T-cell lines with 2uM AZT, 0.5uM ddC, or 5uM ddA. These concentrations of ddN are somewhat higher than those required to protect M/M; however, caution must be used in making stringent comparison between M/M and H9, because different strains of virus were used in different cells. At day 21 there was a viral breakthrough in H9 cells with AZT, even at the highest dose tested (10uM), in agreement with results previously reported by our group and others.

No toxicity (as assessed by trypan blue exclusion) was observed in M/M (prepared by any of the 3 methods) exposed for 14 days to up to 20uM AZT, 2uM ddC, and 100uM ddA; thus the doses of ddN that induced viral inhibition in M/M are much lower (at least 20-200 times) than those necessary to kill the target cells. Also, it is unlikely that the antiviral effect of ddN is an artifact of drug toxicity. Thus, although there were some differences between fresh and 5-day-adherent M/M, both populations could be protected against HIV infection with each of the dideoxynucleoside congeners tested.

In order to better define the basis of this antiviral activity, we evaluated the anabolic phosphorylation of AZT and ddC in M/M and compared it to the phosphorylation in H9 T-cells. In addition, we examined the pool size of the endogenous normal deoxynucleoside-5'-triphosphates (dNTP), 2'-deoxythymidine-5'-triphosphate (dTTP) and 2'-deoxycytidine-5'-triphosphate (dCTP) in each of these cell populations. Similar levels of ddCTP were produced in fresh-elutriated M/M and H9 cells exposed to 0.5uM of ^{3}H-radiolabeled ddC for 24 hours, while 5 day-adherent M/M cells accumulated slightly lower levels of ddCTP. In addition, fresh elutriated M/M and H9 cells had comparable activity of deoxycytidine kinase (dC-Kinase), the enzyme that catalyzes the first phosphorylation of ddC; 5 day-adherent M/M expressed somewhat lower dC-kinase activity. Since ddCTP is thought to compete with the normal nucleoside triphosphate, dCTP, at the level of viral reverse transcriptase, we further examined the level of dCTP in these cell populations. The levels of dCTP in either M/M population were less than 20% of that in H9 cells. Thus, the protective effect of ddC in M/M, in spite of somewhat reduced ddCTP formation, may have been produced by a substantially lower competing dCTP pool size in these cells.

When AZT metabolism was examined in these cells, a somewhat different pattern was seen. Both fresh elutriated- and 5 day-adherent M/M exposed to 10uM ^{3}H-AZT for 24 hours had substantially reduced levels of AZTTP as compared to H9 T-cells. Also the levels of thymidine kinase (dT-kinase) activity were markedly reduced in the M/M cells as compared to the H9 T-cells. However, when dTTP was assayed, both M/M populations had less than 5% of the concentration of dTTP found in H9 T-cells. Thus, even though AZT is phosphorylated less efficiently in M/M than in H9 T-cells, there are substantially reduced levels of the competing dTTP in M/M; as a result, the ratio of AZTTP to dTTP is roughly similar in M/M to that in H9 T-cells.

The ddA metabolism was not evaluated because of the technical difficulty in accurately measuring the final product of ddA anabolism, 2',3'-dideoxyadenosine-triphosphate (ddATP). However the concentrations of endogenous 2'-deoxyadenosine -5'-triphosphate (dATP) in both fresh and 5 day-adherent M/M was <.5 pmole/10^6 cells, compared to 17.35 pmole/10^6cells in H9 cells. Thus, in the case of ddA as well, a small pool size of the competing deoxynucleotidetriphosphate in M/M may permit anti-HIV activity of ddA even if its level of phosphorylation is low.

DISCUSSION

It has become increasingly apparent that infection of cells of the monocyte/macrophage lineage by HIV plays an important role in the pathogenesis of AIDS. they could persistently produce HIV. Because of this, they could serve as a reservoir of HIV and in this context frustrate certain antiretroviral chemotherapies. In addition, since HIV-envelope-induced syncytia formation can lead to T-cell death without the need for such T-cells to become infected per se, HIV-producing macrophages could theoretically cause T-cell depletion even if the T-cells are rendered virally resistant by drugs. Finally, monocyte/macrophages are an important target cell for HIV in the brain and thus may play a role in the pathogenesis of HIV-induced dementia. For these reasons, it is crucial that an anti-viral strategy for HIV infection somehow address the infection of monocyte/macrophages by the virus.

Mature monocyte/macrophages have a limited proliferative potential and certain studies of these cell populations have suggested that they may phosphorylate nucleosides poorly as compared to dividing lymphocytes. In agreement with previous studies of others, we found in these experiments that the phosphorylation (metabolic activation) of at least one dideoxynucleoside analogue, AZT, was substantially reduced in monocytes; however, the metabolic activation of another dideoxynucleoside, ddC, was roughly comparable to that in T cells. It was therefore somewhat surprising that HIV-replication could be suppressed in monocyte/macrophages by each dideoxynucleoside tested (including AZT) at concentrations comparable to those at which the drugs inhibit HIV in T-cells. To further explore this apparent incongruity, we examined the level of dTTP and dCTP in the monocyte/macrophage populations, and found that the level of dCTP was 6 to 8 times lower than that in T-cells, and the level of dTTP was substantially lower (30-35 times). Thus, in the monocyte/macrophage populations, the ratio of the dideoxynucleoside triphosphates to the corresponding normal nucleoside triphosphates was equal or greater than that found in T-cells. There are two known mechanisms by which dideoxynucleosides, as triphosphates, inhibit the replication of HIV (and other retroviruses). One is that they act as competitive inhibitors of the corresponding nucleoside triphosphates at the level of HIV-DNA polymerase (reverse transcriptase) with K_i's of 0.04 to 0.2 uM. The major cellular DNA polymerase (DNA polymerase alpha) is rather resistant to these drugs. In addition, because of the replacement of their 3'-hydroxy(-OH) group with hydrogen or another group, once these compounds are incorporated at the end of a growing chain of viral DNA, no further nucleotide residues can be added. Thus they can act as chain terminators after having elongated a growing chain of DNA by one residue. It therefore follows that the activity of ddN in a given cell will be heavily affected by the ratio of the dideoxynucleoside triphosphate level to the normal endogenous nucleoside triphosphate level, and this may account for the activity of AZT in monocyte/macrophages in spite of the limited phosphorylation of this drug.

Monocytes/macrophages are an heterogeneous population, and one must be cautious in extrapolating the results here to other populations of these cells. Certain subsets of M/M and/or M/M belonging to the same subpopulation but at different stage of maturation could exibit variability in HIV replication, drug activation, or both. We have tried in part to address this by examining monocyte/macrophages prepared by several methods (including cells allowed to adhere and differentiate for 5 days). Nevertheless, it is still possible that HIV-replication in certain differentiated macrophages may be inhibited only by higher concentrations of ddN, and that not all cells within our heterogeneous populations are rendered virally resistant by these agents.

In this regard, it is worth pointing out that Richman and his co-workers in San Diego recently have reported that AZT, ddC and ddA failed to inhibit HIV-infection of monocytes prepared by 3- to 7-day adherence. It is possible as well that subtle differences may have affected the monocyte/macrophage populations in these two studies and influenced the results. It has been shown that monocyte/

macrophages digesting cellular debris, for example, normally excrete nucleosides (such as thymidine). Thymidine can outcompete (nullify) the antiviral activity of AZT. At the same time, following anabolic phosphorylation, thymidine can potentiate the activity of ddC in part by complex allosteric effects acting at the level of ribonucleotide reductase to reduce cellular dCTP pools. It is thus conceivable that in crowded cultures of macrophages actively phagocytosing cellular debris, there may be a variable pattern of antiviral effect induced by dideoxynucleosides, leading to different observations and conclusions. Finally, our current data do not address subtle perturbations of accessory-cell function which theoretically could occur even though an ongoing state of viral replication did not exist. Nevertheless, taking all factors into account, we disagree with the conclusion athat monocytes are drug-resistant reservoirs for these drugs.

Our results suggest that HIV-replication in fresh peripheral blood monocyte/macrophages can be efficiently suppressed by AZT, ddC, or ddA, and that certain differentiated (5-day cultured) populations can also be rendered resistant to viral replication. It is unlikely that these results were due to contaminating T-cells in the populations because 1) our populations contained <1% T-cells, 2) we did not stimulate the cells with IL-2 or PHA, and 3) we used a monocytotropic strain of HIV which infects T-cells less efficiently than M/M. The results suggest at a minimum that the peripheral blood monocytes, which may disseminate HIV through the immune system, can be protected against HIV infection by ddN under some conditions. These results may also have implications in understanding the effects of these drugs on virus in the central nervous system. We have found that HIV-induced dementia may be reversed in certain patients by the administration of AZT, and these observations are discussed in previous annual reports and other sections of this report. It is possible that the protection of monocyte/macrophages in the central nervous system (e.g. microglial cells) by AZT may have contributed to the clinical improvement in these patients.

As a final point, however, it should be re-emphasized that dideoxynucleosides do not substantially reduce HIV expression in T-cells which are already infected. We do not know how long infected monocyte/macrophages produce virus _in vivo_, but such long-lived infected cells may pose a problem for antiretroviral therapy even if _de novo_ infection and recruitment of new cells can be blocked. Further study of the patterns of infection of monocyte/macrophages _in vivo_ will thus be important in designing therapeutic strategies for the antiviral therapy of HIV infection.

PUBLICATION

1. Perno C-F, Yarchoan R, Cooney DA, Hartman NR, Gartner S, Popovic M, Hao Z, Gerrard TL, Wilson YA, Johns DG, Broder S. Inhibition of human immunodeficiency virus (HIV-1/HTLV-III$_{Ba-L}$) replication in fresh and cultured human peripheral blood monocyte/macrophages by AZT and related 2',3'-dideoxynucleosides. J Exp Med, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07254-01 CO

PERIOD COVERED
October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dextran sulfate suppression of viruses in the HIV family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The first step in the infection of human T lymphocytes by human immunodeficiency virus type 1 (HIV-1) is attachment to the target cell receptor, the CD4 antigen. This step may be vulnerable to attack by antibodies, chemicals, or small peptides. One of the goals of our therapy research effort is to define drugs which attack HIV at multiple steps of its life cycle, including the initial binding step. Dextran sulfate (molecular weight approximately 8,000), which has been given to patients as an anticoagulant or antilipemic agent for more than two decades, was found to block the binding of virions to various target T lymphocytes, to inhibit syncytia formation, and to exert a potent inhibitory effect against HIV-1 in vitro at concentrations that could conceivably be attainable in human beings. This drug also suppressed the replication of HIV-2 in vitro. These observations could have theoretical and clinical implications in the strategy to develop drugs against HIV types 1 and 2.

Dextran sulfate, a long chain polymer of glucose, with a molecular weight of approximately 8,000, containing 17-20% sulfur, has been given orally to human beings for more than two decades as anticoagulant or antilipemic agent. This agent was recently shown to be a potent agent against human immunodeficiency virus type 1 (HIV-1) in vitro. We have now investigated the possible mechanism of action of dextran sulfate against HIV-1 and related viruses.

When susceptible interleukin 2 (IL-2)-dependent helper T-cells, ATH8 cells, were exposed to HIV-1 in the form of cell-free virions, essentially all ATH8 cells were destroyed by the cytopathic effect of HIV-1 by day 7 in culture. However, when ATH8 cells were cultured in the presence of more than 1.25 uM dextran sulfate, the target ATH8 cells were completely protected against the cytopathic effect of the virus and their growth was comparable to that of the virus-free control ATH8 population. Dextran sulfate may have two sulfate groups per glucose unit and these sulfate groups appear to be associated with its antiviral activity. Indeed, non-sulfated dextran with a molecular weight of 9,400 showed no protective effect on ATH8 cells against the virus even at 125 uM. Dextran sulfate also protected normal (tetanus-toxoid-specific) helper/inducer clonal T-cells (TM11 cells) against the cytopathic effect of the virus. At concentrations of 0.625 uM to 12.5 uM, this compound gave complete protection and did not suppress the growth of these normal T cells. At similar concentrations, dextran sulfate also inhibited the infectivity and cytopathic effect of human immunodeficiency virus type 2 (HIV-2), another human retrovirus which may cause an AIDS-like disease.

As an additional index of the antiretroviral effect of dextran sulfate, we looked for the presence of proviral DNA in susceptible ATH8 cells continuously exposed to HIV-1 in culture but protected by dextran sulfate. In the absence of the drug, proviral DNA was first detected on day 2, and on day 4 an abundant amount of proviral DNA was detected. In contrast, in ATH8 cells similarly exposed to the virus and cultured in the presence of 2.5 uM dextran sulfate, no proviral DNA was detected throughout the study.

To investigate the mechanism of anti-HIV effect of dextran sulfate, we studied the effect of dextran sulfate on the viral DNA polymerase (reverse transcriptase, RT) activity, using a synthetic template (6). Dextran sulfate showed a potent inhibitory activity against purified HIV-1 RT at $\geq$1 uM, and the RT-mediated DNA synthesis was completely inhibited. This compound, however, at similar concentrations also suppressed the activity of mammalian DNA polymerase alpha, an enzyme that has key DNA synthetic and repair functions in cells. Furthermore, the addition of a nonenzyme protein, bovine serum albumin, readily nullified the RT inhibition by dextran sulfate. In view of these results, the capacity of dextran sulfate to block the infectivity of HIV without cellular toxicity might be difficult to attribute to selective inactivation of viral RT per se.

The initial event in the infection of target cells by HIV-1 is the attachment of the HIV-1 envelope glycoprotein to the CD4 cellular protein. Such an attachment may be necessary, but not automatically sufficient, for effective binding and subsequent penetration. The cytopathic effect of HIV-1 is mediated, at least in part, by an interaction between the gp120 and CD4 molecule that brings about syncytia formation. A surface autofusion phenomenon that destroys the integrity of the cell membrane could also be related to the cytopathic effect of HIV-1.

However, syncytia formation need not necessarily be the only mechanism of cytopathic effect of HIV. Indeed, when susceptible ATH8 cells are exposed to HIV-1 in the form of cell-free virions, most ATH8 cells are destroyed without forming detectable syncytia. Fusion events are thought to be mediated by sequences in the gp41 portion of the envelope. The integrity of tertiary complexes of viral envelope protein is likely to be crucial for these processes. Dextran sulfate might function at any of these points. To determine if dextran sulfate could inhibit syncytia formation, we used a system permitting $CD4^+$ uninfected cells to interact with viral protein expressed on the surface of HIV-1-infected cells. When $CD4^+$ ATH8 cells were cocultured with HIV-1-infected H9 cells, substantial numbers of syncytia were formed in 48 hours. Syncytia were still formed in the continuous presence of 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxyadenosine, retroviral DNA-chain terminators known to be effective against HIV-1 replication at the stage of reverse transcription, but not thought to affect viral binding. Dextran sulfate (6.75 uM), however, completely inhibited syncytia formation between ATH8 cells and HIV-1-infected H9 cells.

Fusion of uninfected $CD4^+$ cells with HIV-infected cells can be blocked by certain antibodies to CD4. In the absence of such antibodies, the syncytia produced die shortly after formation and the surviving cells can be comprised of resistant $CD4^-$ cell. To determine whether dextran sulfate modulates or down-regulates CD4 antigen, we examined the amount of CD4 antigen expressed on normal helper T cells (TM11), susceptible helper T cells (ATH8), and relatively permissive cells (H9) by an immunofluorescence technique using OKT4A antibody. We found no significant change in the CD4 immunofluorescence intensities in any of these target T-cells after exposure to dextran sulfate.

This suggests that dextran sulfate might inhibit the step of the virion binding to the target T-cells. [5-^{3}H]-Uridine-labeled HIV-1 virions derived from the molecular clone HX10 were incubated with uninfected H9 cells in the presence or absence of various concentrations of dextran sulfate, and the radioactivity of virions bound to the cells was determined. We assumed that the capacity of certain compounds to block that amount of virion binding which is inhibitable by OKT4A is also essential for blocking the interaction of gp120 and CD4 protein, thereby blocking infection of HIV. To express the level of virion binding inhibition displayed by various compounds, we calculated the specific inhibition in such a way that the amount of labeled virions bound in the presence of a supermaximal concentration of OKT4A (50 ug/ml) represented 100% specific inhibition, and the amount bound in the absence of drug or antibody represented 0% specific inhibition. By analysing the bound radioactivity on a per-cell basis, we generally found 500 to 2,000 virus particles bound to a cell in the absence of drug under the conditions used. Similar data have been obtained with Jurkat cells and Molt-3 cells and with a different molecularly cloned virus strain WMJ-1. At comparable concentrations of antibody to CD8 (OKT8) we observed almost no inhibitory effect on viral binding. Dextran sulfate completely inhibited the binding of radiolabeled virus to H9 cells. The level of binding inhibition by dextran sulfate was equal to the maximum inhibition achieved by OKT4A at concentrations as low as 1 uM. Nonsulfated dextran (M.W. 9,400), which is inactive against HIV-1, again showed no inhibition. Suramin, a compound (molecular weight 1,429) with six sulfate groups, suppresses the infectivity and replication of HIV-1 at 35 uM *in vitro*, but even at 140 uM this compound did not completely inhibit virion binding, suggesting that this polyanionic substance inhibits HIV-1 by other mechanism(s).

In our [5-^{3}H]uridine-labeled virus binding assay, binding inhibition has been only modest in sera from a number of HIV-1-infected patients and has not been found in all sera showing HIV neutralizing activity. It might be possible that a CD4 contact site of gp120 represents an immunologically tolerated epitope, or is hidden by another portion of the virion envelope.

At least two viral structural determinants are involved in the process of HIV penetration resulting from fusion of viral and cellular membranes and the giant cell-inducing activity of HIV: the CD4 binding site within gp120 and a fusogenic domain believed to reside within the transmembrane *env* protein of HIV, gp41. Our current data suggest that dextran sulfate is capable of inhibiting virion attachment and fusion-dependent events, although other mechanisms (e.g. interference with retroviral uncoating) could be involved in its anti-HIV effect. The way in which dextran sulfate affects virion binding is not established and will require further research.

Our work to date suggests that the anti-retroviral effect of dextran sulfate is not unique to that molecule *per se*, but rather it represents an activity that is common to a broad family of polyanionic polysaccharides and glycosaminoglycans found in nature. We are studying a variety of structure activity relationships of such compounds.

PUBLICATION

1. Mitsuya H, Looney DJ, Kuno S, Ueno R, Wong-Staal F, Broder S. Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD-4$^+$ cells. Science 1988;240:646-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 07202-05 BDMS

PERIOD COVERED

October 1, 1987 through September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Biostatistics and Data Management Section

PRINCIPAL INVESTIGATOR *(List othar professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The Section is the statistical and data management component of the Clinical Oncology Program (COP). The Section provides statistical leadership and data management consultation for major activities of the Program, and is involved in the design, conduct, monitoring, and statistical analyses of intramural and national multicenter clinical trials of experimental treatments for cancer. Other major collaborative efforts include studies to identify important prognostic and treatment selection factors, evaluate diagnostic procedures, develop improved staging systems, and assist investigators in the design, execution, and analyses of major in vitro drug testing studies. The Section develops new statistical designs and biometric methods related to the development and evaluation of new cancer treatments. The Section maintains computerized data collection systems for intramural and national multicenter clinical protocols, and it works closely with interested branches to improve data recording and retrieval. The Section is working to develop specialized clinical data bases for individual branches within the COP. The Section works with the Clinical Center Medical Information System team, allowing COP input into decisions which directly impact patient care and protocol management. The Section assists the Deputy Clinical Director to insure adequate monitoring of protocols through the MIS Toxicity screens and other mechanisms.

1. Collaborative Projects Within Clinical Oncology Program

Members of the Biostatistics and Data Management Section provide to the intramural clinical research program both biostatistical and data management expertise. Our efforts in these areas are described in Sections A) and B) below.

A. Members of the Biostatistics and Data Management Section (BDMS) participate in the development of new protocols and the interim monitoring and data collection for ongoing studies. A member of the Section also serves on the Clinical Research Sub-Panel to review all intramural clinical trials. BDMS staff collaborate in clinical and laboratory studies to evaluate prognostic and treatment selection factors and elucidate tumor biology. The Section provides statistical support for the COP as well as advice on the best ways to use available NIH computer systems or microprocessor based professional workstations for clinical and laboratory research. The Section is also presently developing extensive microcomputer based data management systems for several branches in the COP.

A detailed list of COP projects to which members of the Section have provided statistical input follows:

(1) Performed two interim analyses of ALL (leukemia) protocol 77-02, a cooperative study with 181 patients at five institutions.
(2) Performed two interim analyses of two ALL protocols for average and high risk patients; the high risk protocol is a single arm extension of the successful chemotherapy only (no cranial irradiation) arm on the multi-institutional 77-02 protocol, with modifications in Ara-C administration to prevent CNS relapse and to more aggressively treat systemic disease. The average risk protocol is a randomized extension of 77-02, comparing two chemotherapy only arms -- one with high dose methotrexate and one without.
(3) Performed interim analysis of four phase II trials of 6MP in 85 patients at over a dozen institutions participating in the Pediatric Oncology Group.
(4) Arranged for randomizations and eligibility checklists for protocols to be conducted through Radiation Oncology Branch and Medicine Branch.
(5) Served as member on Institutional Review Board.
(6) Performed major update of results on all soft tissue sarcoma protocols, comparing adjuvant chemotherapy to no chemotherapy in patients with extremity tumors, and with head, neck and trunk tumors, comparing limb-sparing surgery to amputation in patients receiving adjuvant chemotherapy, comparing a short course adjuvant chemotherapy regimen (350 mg/m^2 doxorubicin) with standard course (550 mg/m^2); and comparing radiation to no radiation in patients with high grade soft tissue sarcoma of extremities with local surgical resection or with low grade soft tissue sarcoma of head, neck and trunk, or extremities.
(7) Performed analyses of data regarding quality of life issues in patients with soft tissue sarcomas.
(8) Analyzed prognostic factors and treatment effects in patients with small cell lung cancer.
(9) Performed analyses to evaluate effects of perioperative transfusions in patients treated for colorectal hepatic metastases.
(10) Determined the association between immunohistochemical and clinical

findings in patients with lung cancer of various pathologic types.
(11) Examined effects of interleukin-2 on renal function in patients with advanced cancer.
(12) Performed updated analysis of a study comparing standard with aggressive therapy for the treatment of mycosis fungoides.
(13) Prepared statistical design of a study evaluating whether mesna can control bleeding in patients treated with cyclophosphamide.
(14) Prepared statistical considerations for a trial involving treatment of hairy cell leukemia patients.
(15) Developed design for a trial evaluating GM-CSF in pediatric patients.
(16) Performed analysis of in-vitro drug sensitivity testing.
(17) Developed design for a study involving chemotherapy and bone marrow transplantation in patients with lymphoma.
(18) Analyzed data from a study of patients with astrocytomas treated with radiation.
(19) Analyzed data from a study of pediatric patients who received radiation for chest wall sarcomas.
(20) Determined whether it was permissible to terminate a randomized trial early on the basis of equivocal results.
(21) Performed analyses of the randomized early stage breast cancer study.
(22) Performed a retrospective analysis of data from a study of patients with prostate cancer.
(23) Performed analysis of testicular function in patients following radiation.
(24) Performed analyses of data for a study of glucose production.
(25) Performed analysis of prognostic factors in an esophageal cancer trial comparing chemotherapy versus surgery alone.
(26) Performed analyses to predict outcome of patients receiving combination chemotherapy for treatment of fever.
(27) Analyzed data on amelioration of dermal photosensitivity in mice.
(28) Delivered a lecture on statistical considerations in cancer clinical trials for the COP's combined rounds lecture series.
(29) Synthesized articles on meta-analysis, and provided advice to COP investigators regarding use of this technique in a clinical research setting.
(30) Determined that the current trial comparing radiation to no radiation for treatment of high grade soft tissue sarcomas should remain open for further patient accrual.
(31) Performed analyses of the Stage III metastatic breast cancer study.
(32) Analyzed effects of catheters in fever treatment protocols.
(33) Provided advice regarding interpretation of sophisticated statistical techniques used to analyze a study of the relationship between weight loss and wound healing strength in mice.
(34) Examined the relationship between depression in parents of children with AIDS and parents of children with cancer.
(35) Performed updated analysis and identified prognostic factors in a trial of patients with extensive small cell lung cancer.
(36) Performed analyses of data for patients receiving IL-2±LAK to determine whether prognostic factors can be identified for those patients who exhibited substantial clinical responses to therapy.
(37) Developed design of three clinical trials involving use of IUdR.
(38) Performed analyses of data pertaining to rehabilitation in women

participating in the randomized early stage breast cancer trial.
(39) Provided advice and guidance to Pediatric Branch Medical Staff Fellows regarding survival analyses in clinical trials.
(40) Performed analyses of data for a study relating procedural pain and type of anesthesia administered to pediatric oncology patients.
(41) Provided additional analyses on a study involving computerized tomographic prediction of extraluminal spread and prognostic indicators of lesion width in esophageal cancer.
(42) Studied the effects on a clinical trial of the increasing proportion of women noted in recent years.
(43) Analyzed data from patients with pheochromacytoma to try to predict whether such tumors are benign or malignant.
(44) Analyzed data to demonstrate lack of synergy between VP-16 and cisplatin.
(45) Developed design for a study of multi-modality treatment for advanced breast cancer.
(46) Prepared and delivered an intermediate-level talk on statistical considerations in analysis and interpretation of cancer clinical trials.
(47) Provided consultation to an investigator on a randomized trial of total parenteral nutrition in patients undergoing surgery.
(48) Performed analyses pertaining to prognostic factors in soft tissue sarcoma of the extremities.
(49) Performed analyses of a randomized trial in metastatic breast cancer.
(50) Studied the relationship between gene expression, amplification, and clinical data in patients with breast cancer.
(51) Provided statistical considerations for a pilot study of intensive induction therapy and autologous bone marrow transplantation for the treatment of high grade lymphomas.
(52) Designed a clinical trial to evaluate the effect of cytologic changes associated with immunotherapy.
(53) Analyzed factors in survival among patients with a thoracotomy for pulmonary metastases following treatment for a soft tissue sarcoma primary tumor.
(54) Provided a statistical considerations description for the manual, "How to Write a Protocol", being prepared for use by the NIH Clinical Center.
(55) Analyzed a study of salvage treatment with intermediate dose methotrexate and 5FU in metastatic breast cancer.
(56) Provided advice to an investigator regarding the design of a trial of adult soft tissue sarcoma patients.
(57) Refereed a manuscript submitted to Cancer Research at the request of a COP investigator who is an associate editor of the journal.
(58) Provided advice to an investigator regarding the design of a trial comparing bactrim to pentamadine for prevention of PCP pneumonia in patients with Hodgkin's disease.
(59) Analyzed factors associated with liver toxicity in patients treated with interleukin-2.
(60) Performed survival and response analyses of patients randomized to three IL-2 trials: advanced cancer, adjuvant colorectal hepatic metastases, and adjuvant melanoma. Provided recommendations with respect to additional accrual for each of these studies.
(61) Participated in analysis of psychosocial impacts of alternative breast cancer therapies.

(62) Performed analyses relating immunohistochemical markers, gender, smoking history, and particular lung cancer tumor histologies.
(63) Provided analyses for a study of mastectomy following intensive chemotherapy for locally advanced breast cancer.
(64) Analyzed a study of a large retrospective series of data collected on patients with prostate cancer treated with radiation since 1970.
(65) Developed design for a Phase II clinical trial in colon cancer.
(66) Developed a design for a Phase I/II trial in ovarian cancer.
(67) Performed analysis of prognostic factors for the randomized IL-2/IL-2+LAK protocol for patients with advanced cancer.
(68) Performed analysis of a study of oncogenes in cell lines.
(69) Provided advice to investigators regarding analysis of toxicity data from a trial of patients with lymphoma.
(70) Provided advice to an investigator regarding analysis of complications from a randomized trial of intraoperative radiation therapy versus control in several disease sites.
(71) Performed analysis of data from laser light scattering experiments in a thoracic model.
(72) Performed updated analyses of a study of patients with locally advanced breast cancer.
(73) Analyzed data pertaining to dose intensity in a breast cancer protocol.
(74) Analyzed data from an animal experiment associating cachectin activity level with other factors.
(75) Advised a pharmacist for the COP regarding design and analysis of experiments involving stability of Bactrin over time.
(76) Analyzed data from the male breast cancer study.
(77) Performed preliminary analysis of data from a retrospective study of aplastic anemia patients and development of infection.
(78) Performed preliminary analysis of a study of the relationship between ceruloplasmin and outcome in patients with locally advanced breast cancer.
(79) Performed updated analysis of three protocols for treatment of osteosarcoma.
(80) Analyzed a phase II study of high dose carboplatin and diethyldithiocarbanate chemoprotection in the treatment of relapsed ovarian cancer.
(81) Prepared statistical design considerations for a pilot study of Promace-CytaBom for the treatment of grade I, II, and III angiocentric immunoproliferative lesions.
(82) Provided advice to an investigator regarding application of a complete program for the fitting of cell survival curves.
(83) Provided advice regarding the design of a trial of intermittent AZT.
(84) Developed a design for a pilot study involving AZT + GM-CSF.

B. Data Management Activities

The Section has continued the development and maintenance of several systems which facilitate the monitoring of protocols:

(1) A comprehensive data management system has been implemented and is being augmented for monitoring all immunotherapy protocols.
(2) The CAPRI system, the primary COP data base, has been continuously updated.
(3) Existing data management systems were analyzed and new microcomputer systems

for the Medicine, Pediatric and Metabolism Branches are being designed, developed and programmed.
(4) A variety of graphs, plots and other statistical reports have been provided to COP branches.
(5) In support of the COP use of personal computers, assistance and consultation was provided in the selection and installation of hardware, evaluation of software packages, and designing and implementing scientific PC programs.

A detailed list of data management projects undertaken by members of the BDMS for the COP follows:

(1) Collection, maintenance, and reporting of basic survival and protocol entry data on COP active patients.
(2) Data management, programming, retrievals and analyses as required by all branches of the COP.
(3) Development of data collection forms, as required, for all branches of the COP.
(4) Create, modify and update data bases for researchers of the COP.
(5) Maintenance of a computer file of the latest actual outpatient clinic visit for all active patients.
(6) Support to insure that all patients receiving chemotherapy, especially investigational drugs, have a valid Clinical Center protocol number for pharmacy records.
(7) Maintenace of various computer packages used by the Section.
(8) Collaboration with Clinical Center computer staff on abstracting MIS data for protocol uses, and on computerized hospital data collection.
(9) Automatic printing of all data from the MIS Toxicity and Protocol Monitoring System as Progress Notes and distribution of these documents to appropriate investigators.
(10) Assist research nurses and principal investigators in using personal computers for protocol data management.
(11) Provide branches with access to computer programs to do standard statistical analyses.
(12) Evaluation of software packages for the IBM-PC.
(13) Maintain major statistical and plotting programs for PC to allow BDMS statisticians to perform Kaplan-Meier curves, logrank tests (including stratified version), logistic regression, and Cox regression.
(14) Computer needs analysis and equipment and software purchase recommendations for acquiring personal computers; training staff in the use of PCs.
(15) Provided data management support for all immunotherapy protocols through initiation and/or completion and maintenance of the ten computer forms using prospective and retrospective review of office charts and medical records.
(16) Determined completion status of each of the ten computer forms for all immunotherapy protocols and manually provided lists of those patients to the staff responsible for completing the forms.
(17) Assisted the senior staff in measuring and recording evaluable tumors for all immunotherapy patients.
(18) Submitted for data entry all immunotherapy forms, proofread and corrected all data entered, and provided updates of the Immunotherapy Master List.
(19) Early extensive support to the Radiation Oncology Branch and the Early Stage Breast Cancer Protocol, including the preparation of programs, graphs and

reports.
(20) Continued support of COP randomization activities with the addition of two new protocols and the revision of several existing studies.
(21) Developed and implemented patient tracking systems for the Pediatric and NCI-Navy Medical Oncology Branches.
(22) Provided extensive support to the Psychological Testing Component of the Pediatric Leukemia protocols.
(23) Continued to support the Memorial-Sloan Kettering System on the Data General at the NCI-Navy Medical Oncology Branch, including system maintenance and documentation.
(24) Acted as a coordinating center for five multi-institutional clinical trials in ovarian cancer and pediatric leukemia, involving data collection, data processing, analysis and report production.
(25) Development of microcomputer-based Research Protocol Data Base for the Medicine Branch using the 4th Dimension data base program application on the Apple Macintosh.
(26) Development, programming and testing of Pediatric Data Base Management System using dBaseIII+ on the IBM PC.
(27) Development, programming and testing of Metabolism Data Base Management System using Foxbase+ on the IBM PC.
(28) Installed a modified version of the Pharmacy System used by the NCI-Navy Medical Oncology Branch for the Clinical Center Outpatient Pharmacy servicing COP patients on an IBM PC.
(29) Downloaded data from the IBM mainframe to the PC with accompanying documentation.
(30) Conducted interviews with branch personnel and assisted in the completion of questionnaires by branch chiefs and research nurses concerning data management support and needs in each branch.

2. Projects Outside COP

A. The BDMS also participates in biometric activities outside of the COP. A detailed list of projects outside of COP in which the Section's statisticians have provided statistical input include the following:

(1) Advised member of a Clinical Center panel regarding design of a study evaluating personnel attitudes and knowledge about confidentiality of hospital records.
(2) Reviewed a protocol for its statistical considerations, for the Clinical Center Nursing Department.
(3) Consultation for an NICHD physician regarding analysis of data from a study evaluating sperm motion.
(4) Served as a referee for two manuscripts submitted to Blood.
(5) Reviewed a paper on clinical trials methodology for a statistician from NINCDS.
(6) Reviewed and critiqued a manuscript on tendon/ligament calcifications following isotretinoin use for various skin diseases, at the request of an investigator in Dermatology Branch, NCI.
(7) Provided statistical advice on analysis and design of trials in hairy cell leukemia and in lymphomas for investigators from the BRMP, DCT, NCI.
(8) Analyzed prognostic factors in patients with Hodgkin's disease, treated in

Spain, for staff of the Clinical Center Pathology Department.
(9) Provided consultation to an investigator for NICHD regarding analysis of interactions between time and hormone release.

B. In addition to data management support for intramural trials, the BDMS provides data management services outside the COP. Project staff have provided Operations and/or Statistical Center support to a number of multi-institutional extramural trials. This support includes performing randomizations, design of data collection instruments, software design and development, production of regular status reports, and production of ad hoc reports and tabulations as directed by the study statistician. The extramural trials supported include:

(1) 7601/7602, Treatment of Early Stage Ovarian Cancer
(2) CCSG-191P, CCSG Protocol for Acute Lymphoblastic Leukemia
(3) CCSG-134P, CCSG Protocol for Poor Prognosis Acute Lymphoblastic Leukemia
(4) CCSG-144P, CCSG Protocol for Average Prognosis Acute Lymphoblastic Leukemia

3. Biometric Research

Current biostatistical research being conducted includes:

(1) A two stage method for selecting interactions between variables to be evaluated for prognostic importance.
(2) Development of data management systems which may serve multiple purposes.
(3) Development of estimation methods for parametric transformations in survival analyses.
(4) Development of two-stage estimation models in carcinogenesis.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06119-19M

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytogenetic studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jacqueline Whang-Peng	Senior Investigator	MB	NCI
Others:	Turid Knutsen	Med. Technologist	MB	NCI
	Elaine Lee	Med. Technologist	MB	NCI
	Yi-Fa Liu	Visiting Fellow	EEB	NCI
	Yuan Su	Visiting Fellow	MB	NCI

COOPERATING UNITS *(if any)* Environmental Epidimiology Branch, NCI
Medical Oncology Branch, Naval, NCI. Surgery Branch, NCI,
Clinical Pharmacology Branch, NCI. Pediatric Oncology Branch, NCI
Laboratory of Tumor Virus Biology, NCI.

LAB/BRANCH

Medicine Branch

SECTION

Cytogenetic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Cytogenetic studies of human neoplastic, hematologic, and congenital diseases, with special emphasis on patients with AIDS who develop leukemia, lymphoma, or Kaposi's sarcoma. Specific diseases studies include lymphoma (Burkitt's and non-Burkitt's), non-small cell carcinoma of the lung, rhabdomyosarcoma, renal cell carcinoma, small-cell tumors in childhood, acute lymphocytic leukemia, preleukemia, secondary leukemia, and esophageal cancer.
2. Cytogenetic studies in rhabdomyosarcoma: direct studies of specimens from 12 patients and studies of 8 established cell lines.
3. Localization of genes in normal chromosomes, using in situ hybridization.
4. Cytogenetic and virus integration studies (using in situ hybridization) of human foreskin cell lines infected with human papilloma virus.
5. Fragile sites studies:
   - A. Continuation of in vitro studies of fragile sites in peripheral blood chromosomes from individuals of different ages.
   - B. Studies of fragile sites in the peripheral blood of high risk cancer families, e.g., melanoma and dysplastic nevi syndrome.

C. Studies of fragile sites and/or SCEs in the peripheral blood of patients with small cell lung cancer and family members in familial renal cell cancer.
6. Use of immunofluorescent labeled antibody to topo II to detect the site of action in HSR (homogeneously staining region).
7. Study of the relationship between topo II and fragile sites. Human lymphocyte chromosomes are labeled with ^3H thymidine and then subject to the fragile site harvesting procedure. The cleavable complexes are trapped with SDS and the protein:DNA complexes analyzed to determine if topo II is the protein involved in these complexes. In the original experiment, the amount of trapped protein was too small to detect the presence of Topo II in the DNA complex. It has been proposed that the fragile site is a nucleosome free region. In collaboration with Dr. Berton Zbar, Ruth Neta, and Juluang Huang the working probes D3S3 and D3S2 (polymorphic alleles to Mspl) will be used.
8. Study of the chromosomal abnormalities in bone marrow and spleen of mice receiving IL-1 treatment before, during, and after lethal dose of X-ray irradiation.

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Berton Zbar	Senior Staff	FCRF	NCI
Ruth Neta	Senior Staff	MOB	Navy
Susan Banks-Schlegel	Senior Staff	LD AD	NHLI
Richard Schlegel	Senior Staff	LTVB	NCI

Susan Naylor	Senior Researcher University of Texas, San Antonio, Texas
James Wong	Director, Dept. of Biochemistry & Molecular biology, Harvard Medical School, Mass.
Leroy Liu	Senior Staff, Dept. of Physiological Chemistry, Johns Hopkins Medical School, Baltimore, Md.
Juluang Huang	Senior Researcher, Molecular Biological Institute, Academia Sinica, Taipei, Taiwan.

Projects completed:

1. The following genes were localized:
   - A. pGTH4 (Glutathione S-transferase gene) at 1p31 (In Press)
   - B. Topo I gene at 20q12-13.2 (manuscript in preparation)
   - C. Topo II gene at 17q21.2-22 (In Press)
   - D. Human neutrophil elastase gene at 11q14 (In Press)
   - E. pA1, pADR1 (adriamycin resistant) at 7q21.1 (published)

2. Proposed origin of small cell lung cancer (SCLC) cells.
Two SCLC patients, who developed erythroleukemia after combined modality chemo-radiotherapy for their lung cancer had cytogenetic studies of their leukemic cells. Both erythroleukemias exhibited the del(3)(p14p23) which is predominantly found in SCLC. In four other of our patients with secondary leukemias associated with other malignancies, no deletions of 3p were found. These findings could be accounted for by one of three possible mechanisms: (1) an inherited recessive gene (anti-oncogene or tumor suppressor gene) in this region of 3p has been uncovered by the combined modality therapy, (2) an inherited predisposition to damage of both chromsomes at 3p14 leading to SCLC and erythroleukemia after exposure to carcinogens and/or chemo-radiotherapy, or (3) lineage specificity for the 3p deletion in SCLC and erythroleukemia suggesting a common bone marrow precursor.

3. Fragile sites and non-specific chromosome abnormalities in hereditary and sporadic cutaneous malignant melanoma. This pilot study quantitated fragile site expression in individuals with familial dysplastic nevi syndrome (DN) or with both familial cutaneous malignant melanoma (CMM) and DN, as well as other individuals with sporadic DN, CMM, and unaffected spouses. No striking association was seen between fragile site expression and sun exposure, caffeine, alcohol intake, or skin type. All categories of familial disease had increased fragile site expression compared to sporadic affecteds and normal controls, in spite of a younger age distribution, age having been associated with fragile site expression in an earlier study.

4. Cytogenetic studies were performed on a total of 30 non-small cell lung cancer and 18 small cell lung cancer patients. The presence of normal and abnormal chromosomes number 3 are listed in table 1 for each disease category. The presence or absence of the specific chromosome 3 abnormality associated with SCLC is also included. We concluded that 27% of the non-small cell lung cancer contained the chromosome 3 marker typical of SCLC and 95% of the small cell lung cancer patients had this chromosome 3 marker in their tumor cells.

TABLE 1

		Chromosome #3		
	No. pts.	Normal	Abnormal	Specific for SCLC
Non-small cell lung cancer				
Squamous cell	4	2	2	1
Adenosquamous	3	0	3	1
Adenocarcinoma	5	5	0	0
Adenocarcinoma, poorly diff.	1	1	0	0
Large cell	1	0	1	0
Bronchoalveolar carcinoma	4	2	2	1
Mesothelioma	3	1	2	1
Mucoepidermoid	1	1	0	0
Neuroendocrine				
true carcinoid	1	0	1	1
neuroendocrine atypical carcinoid	3	0	3	3
non-small with neuroendocrine feature	4	2	2	0
TOTAL	30	14	16	8 (27%)
Small cell lung cancer	18		17	17 (95%)
Extrapulmonary small cell lung cancer	4	2	2	1 (25%)

5. A study of fragile sites and sister chromatid exchange (SCE) in bone marrow (BM) and peripheral blood (PB) of cigarette smokers revealed a significantly increased frequency of SCEs in smokers with a 5 or more cigarette pack year history in both BM and PB. The peripheral lymphoytes of smokers also showed (a) a significantly higher frequency of fragile sites, (b) an increased number of metaphases with extensive breakage, and (c) elevated expression of fragile sites at some cancer breakpoints and oncogene sites.

PUBLICATIONS:

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3. Whang-Peng, J.: Genes, chromosomal translocation, and neoplasia. In Molecular Biology of Neoplasia, E.H. Chang, J-K. Lin, and P.C. Huang (Eds.), Academica Sinica, Taipei, 1985, pp. 243-250.

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findings in the polycythemia vera: long-term follow-up in patients randomized to treatment by the Polycythemia Vera Study Group. In Regulation of Erythropoiesis, Zanjani, E.D., Tavassoli, M., and Ascensao, J.L. (Eds.), Pergamon Press, New York, (1987) (In Press).

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SUBMITTED:

19. Tsai-Pflugfelder, M., Liu, A.A., Tewey, K.M., Whang-Peng, J., Knutsen, T., Huebner, K., Croce C.M., Wang, J.C.: Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome 17q21-22.
Submitted to *PNAS*.

20. Whang-Peng, J., Banks-Schlegel, S.P., Lee, E.C.: Cytogenetic studies of esophageal carcinoma cell lines. Submitted to *Cancer Res*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06513 12 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Pharmacology of Antimetabolite Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name title, laboratory, and institute affiliation)*

Bruce A. Chabner, M.D.	Director, DCT	OD, DCT, NCI
Carmen Allegra, M.D.	Senior Investigator	CPB, DCT, NCI
Keisuke Aiba, M.D.	Guest Researcher	CPB, DCT, NCI
Robert L. Fine, M.D.	Medical Staff Fellow	CPB, DCT, NCI
Patrick Elwood, M.D.	Medical Staff Fellow	CPB, DCT, NCI
Clement Knight, M.D.	Visiting Fellow	CPB, DCT, NCI
Donna Boarman	Biologist	CPB, DCT, NCI
James C. Drake	Biologist	CPB, DCT, NCI

COOPERATING UNITS *(if any)*

NCI-Navy Oncology Branch, COP, DCT, NCI
Critical Care Medicine Department, Clinical Center, NIH
NCI Pediatric Oncology Branch, Clinical Center, NIH

LAB/BRANCH

Medicine Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.5	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

During Fiscal Year 1988, our work continued on the interactions of metabolites with mammalian protozoan cells. Our major projects were the following:

1. Continued work on the mechanism of de novo purine and thymidylate synthesis inhibition by antifolates, including methotrexate, has strengthened a novel postulate that inhibitionof metabolic pathways results from direct enzyme inhibition rather than via an indirect mechanism of folate depletion. These studies illustrates that de novo purine and thymidylate synthesis are inhibited by clinically relevant concentration of methotrexate without significant folate depeltion in two human cell lines (breast MCF-7 and promyelocytic from normal human volunteers. These studies further suggest that the mechanism of metabolic inhibition is through direct inhibitory effects of accumulated dihydrofolate polyglutamates on the folate-requiring thymidylate synthase and AICAR transformylase. This work has been supported by corroborating the relative folate preservation during antifolate exposures using a highly sensitive and specific assay for intracellular folates. This assay was developed by Dr. Priest and is capable of directly measuring folates without the need for radiolabels with their attendant deficiencies.

2. During the course of the above studies, a novel physiologic folate was discovered, 10-formyl-dihydrofolate. Continued investigations have illustrated the formation of this compound in breast cells and in normal human myeloid progenitor cells. Identification and characterization of the enzyme responsible for the formation of dihydrofolate has been accomplished and the effects of this folate on folate-requiring

enzymes has been investigated. The new folate is a potent inhibitor of thymidylate and GAR transformylase but, is an excellent substrate for AICAR transformylase, further supporting the concept that folate depletion is only a minor factor in the mechanism of action of methotrexate.

Studies directed toward a more complete understanding of leucovorin rescue may improve the clinical application of high-dose methotrexate, a strategy capable of overcoming most known mechanisms by which neoplastic cells become resistant to methotrexate. Detailed investigations of this phenomenon have shown that concentrations of folates in vast excess of these found in untreated cells are required to affect cell rescue from methotrexate, suggesting competition at the level of folate-dependent enzymes between reduced folates and direct enzyme inhibitors such as methotrexate and dihydrolate polyglutamates. Further, dihydrofolate reached plateau levels in cells and this event may be interpreted as evidence for competition between dihydrofolate and the antifolate for DHFR activity as an important occurrence in the process of leucovorin rescue.

3. Drug resistance to antimetabolite agents is a major limiting factor in the therapy of malignant diseases. We have investigated the interaction of leucovorin with 5-fluorouracil in multiple colon cell lines developed by Dr. Park at the NCI-Navy Medical Oncology Branch. We have found that leucovorin can markedly enhance the potency of 5-fluorou-racil and 5-fluorodeoxyuridine in selected lines. Currently, we are investigating the mo-lecular mechanism of this interaction and we are investigating the additional mechanisms of resistance in the setting of combination treatment with fluoropryrimidines and leucovorin. Parallel studies are ongoing using tumor samples from patients with breast cancer who are being treated with 5-fluorouracil and leucovorin. Studies with human colon cell lines show that 5-FU resistance in these cells may result from differences in the incorporation of 5-FU anabolites into RNA. The reason for these differences does not appear to be based on differences in activating enzyme levels and the precise explanation are presently under investigation. In contrast, tumors obtained from patients with breast cancer appear to have remarkable changes in both anabolic and target enzyme expression as bases for drug resistance.

In a recently completed trial using 5-FU and leucovorin for the treatment of 54 patients with metastatic breast cancer, we noted a 25% overall response rate in heavily pretreated patients who (90%) failed prior therapy with 5-FU. Serial tissue samples obtained from 20 patients demonstrated a marked enhancement and persistence of thymidylate synthase binding by 5-FU in the presence of leucovorin compared to patients treated without leucovorin. Of interest, 5-FU was noted to produce an acute (24 hours) increase in thymidylate synthase expression (2.5-fold). The mechanisms of this event maybe important in drug resistance and are currently under investigation.

4. Pneumocystis carinii (PC) pneumonia is the most commonly recognized cause of mortality in AIDS patients. Traditional therapy includes the use of the antifolate trimethoprim. Clinically resistant infection and adverse reactions to standard therapies occur in up to 65% of AIDS patients being treated for PC pneumonia. Our laboratory has developed a novel therapy for this infection using trimetrexate, a lipid-soluble antineoplastic antifolate that was found to be 1500-fold more potent than trimethoprim in inhibiting the catalytic activity of PC dihydrofolate reductase. We further found that leucovorin can easily penetrate mammalian host cells and effect

rescue of these cells from the lethal effects of trimetrexate. PC organisms are unable to transport leucovorin and are thus killed by the trimetrexate. These findings were applied to the treatment of PC pneumonia in 49 AIDS patients. The overall response rate, including patients failing conventional therapy, was 70%. We feel that the use of trimetrexate with leucovorin represent represents an important novel approach to the treat ment of this highly lethal and prevalent infection.

In addition to exploiting transport differences between protozoan and host cells, we are searching for more effective antiprotozoan agents by investigating inhibitors of enzymes unique to the protozoan organism such as those of de novo folate synthesis. Dihydropteroate synthase is one such enzyme and presently we have purified and characterized the enzyme from P. carinii and T. gondii. Studies are ongoing to identify potent inhibitors of this target, such as novel analogs of sulfones and sulfonamides which are available from a variety of drug repositories.

5. Recent work has demonstrated that multidrug-resistant human breast and small-cell lung cancer lines have an increased phosporylation of a 20-kD membrane-bound protein. Evidence suggests that protein kinase C is responsible for the phosphorylation, and we have found its activity to be sevenfold higher in resistant breast cancer cells. When pro tein kinase C is activated, it leads to increased multidrug resistance and phosphorylation of the 20-kD protein as well as decreased intracellular drug accumulation. This induced multidrug-resistant phenotype can be inhibited by calcium channel blockers and calm odulin antagonists. We are investigating protein kinase C inhibitors, such as staurosporine and bryostatin, for activity against resistant tumor cell lines. Concomitant with our studies on protein kinase C, we are looking at the phosphatidylnositol (PI) cycle and have found increased activity of this cycle in resistant cells. We have also found arach odonic acid metabolism, and we are attempting to exploit these differences with agents known to inhibit the PI cycle (plant PI) and prostaglandin and leukotriene pathways (SRS-A analogs).

6. The transport of folate and folate analogs such as methotrexate is energy dependent and and probably requires the activity of membrane bound proteins. Such proteins, have been purified from normal and malignant cell and we are in the process of sequencing and characterizing the proteins and generating antibodies for the purpose of assay development. These reagents will also aid in the cloning of the DNA responsible for the protein. We are presently attempting to clone the DNA from the normal and malignant cells. These studies will ultimately provide valuable insights into the prevalence of transport as a mechanism of clinical drug resistance and strategies for circumventing such resistance. In addition, these studies may provide a prospective indication of antimetabolite sensitivity.

PUBLICATIONS

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Allegra, C.J., Curt, G.A., Baram, J., Sholar, P.W., Yeh, G.C. and Chabner, B.A.: Antimetabolites. In: Pinedo, H.M. and Chabner, B.A. (Eds.), Cancer Chemotherapy Annual, 8. Elsevier, Amsterdam, 1986, pp. 1-27.

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B.A. (Eds.): Cancer Chemotherapy Annual, 8. Elsevier-North Holland, Amsterdam, 1986, pp. 117-128.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06516 07 CP

PERIOD COVERED October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Drug Resistance in Human Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Kenneth H. Cowan, M.D., Ph.D.	Senior Investigator	CPB, COP, DCT, NCI
Merrill E. Goldsmith, Ph.D.	Microbiologist	CPB, COP, DCT, NCI
Craig Fairchild, Ph.D.	PRAT Fellow	CPB, COP, DCT, NCI
S. Percy Ivy, M.D.	Biotechnician	CPB, COP, DCT, NCI
Philip Vickers, M.D.	Visiting Fellow	CPB, COP, DCT, NCI
Alan Townsend, M.D.	Biotechnician	CPB, COP, DCT, NCI
Jeffrey Moscow, M.D.	Clinical Associate	CPB, COP, DCT, NCI
Mary Jane Madden, M.D.	Chemist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

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BRMP, FCRC, NCI

LAB/BRANCH Clinical Pharmacology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION NIH, National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This labortatory has been investigating genetic and biochemical changes associated with drug resistance in human tumors. We have characterized an adriamycin resistant human breast cancer cell line which has developed the phenotype of multidrug resistance. Resistance is associated with decreased drug accumulation (2-3 fold) increased activities of glutathione peroxidase (12 fold), glutathione transferase (45 fold), decreased expression of aryl hydrocarbon hydroxylase (cytochrome P1-450). We have isolated cDNA clones from this resistant cell line which encode the gP 170 membrane glycoprotein, a gene which is often, if not always, associated with the development of multidrug resistance. We have also cloned the cDNA for the anionic glutathione transferase which is transcriptionally activated in the Adr^R MCF-7 cells. We have begun a study investigating the expression of the P-glycoprotein gene as well as the expression of several drug metabolizing enzymes including the anionic glutathione transferase GST-π in the development of clinical drug resistance.

A. Multidrug Resistance in Human Breast Cancer Cells

We have been studying the molecular genetic and biochemical changes in multidrug resistant breast cancer cells selected in our laboratory. Resistance in these cells is associated with 2-3 fold decrease in drug accumulation, increased expression of glutathione peroxidase and glutathione transferase activity, and decreased expression of aryl hydrocarbon hydroxylase (cytochrome $P_1$450). These changes are remarkably similar to those which are induced by carcinogens in rat hyperplastic liver nodules and which are associated with resistance to many structurally unrelated hepatotoxins in that system. In addition, an immunologically related aniomic glutathione transferase is induced in both MDR breast cancer cells and in xenobiotic resistant rat hyperplasic liver nodules. These studies suggest that the mechanisms associated with de novo and acquired drug resistance may be the same. Three manuscripts in this work have been published, two additional manuscripts have been submitted.

B. Glutathione S-Transferase

We have recently shown that multidrug resistance in MCF-7 breast cancer cells selected for primary resistance to adriamycin is associated with an increase in glutrothrone S-transferace activity. This increase is due to the induction of the anionic isozyme of GST (GST π). We have purified this isozyme and generated polyclonal antibodies against it. Following affinity purification, this polyclonal antibody reacts with a single protein on western blot analysis. This antibody is being used to screen human tumor specimins for GST π expression using immunohistochemical staining and western blot analysis. Monoclonal antibodies against GST π purified from Adr^R MCF-7 cells have been generated in collaboration with Raphe Kantor at FCRF. These antibodies will be used to screen for different epitopes of anionic GST in order to compare the isozymes produced in different tissues.

Jeff Moscow has isolated GST π cDNA clones from a library constructed by Merrill Goldsmith from Adr^R MCF -7 RNA. This gene code for a 750 bp mRNA which is overexpressed in Adr^R cells. Mary Jane Madden has sequenced this gene and shown that its sequence is remarkably homologous to the rat anionic human GST π gene but differs markedly from the human basic GST genes.

The human GST π cDNA has been used to sreen RNA extracted from normal and malignant human tissues. GST π is expressed in 24/26 human colon cancers but in only 2/10 normal colon tissue samples. This gene may thus represent a marker of de novo resistance to chemotherapy, in this carcinogen-induced tumor. Other studies have shown that GST π expression is increased in 2 patients with recurrent pre B cell ALL relative to the expression in 3 patients with pre B cell ALL at initial presentation. Thus, GST π expression may be a useful marker in acquired resistance to chemotherapy.

We have also found a relation between GST π expression and the absence of estrogen receptors in breast cancer cell lines using Western and Northern blot techniques. Similarly, GST π expression also related inversely to expression of estrogen receptors in primary breast cancer. GST π RNA is moderately high in ER negative ($\leq$10 fmoles lung) breast cancers but is present in low or undeductable levels in ER positive breast cancers. Thus, this gene may be a marker of hormone resistance in breast cancer. Whether this reflects any difference in chemosenitivity is unclear. This work has been accepted for publication in the Proceedings of the National Academy of Sciences.

C. P- Glycoprotein - *mdr* gene

Craig Fairchild, and Percy Ivy, and Merrill Goldsmith have isolated P-glycoprotein cDNA sequences from Adr^R MCF-7 cells. This gene is overexpressed in most multidrug resistant cell lines. Its expression in human tumors is under investigation.

As alluded to previously, studies from our lab have shown that many biochemical changes in MDR MCR-7 cells are similar to those that are associated with xenobiotic resistance in carcinogen-induced rat hyperplastic liver nodules. Further studies have now shown that xenobiotic resistance in rat HNS and hepatomus is associated with overexpression of P-glycoprotein gene. Moreover, acute treatment with acetlyamino-fluorene and partial hepatectomy results in over an 80 fold induction of P-glycoprotein expression. This acute induction of P-glycoprotein represents an useful model to examine the reulation of P-glycoprotein gene expression. This work has been published in the Proceedings of the National Academy of Sciences.

D. Alteration in Hormonal Sensitivity in MDR Breast Cancer Cells

MDR breast cancer cells have become cross resistant to antiestrogens. Phil Vickers in our lab in collaboration with Robert Dickson in the Medicine Branch has examined the hormonal sensitivity of Adr^R MCF-7 cells. In contrast to the parental MCF-7 cells, estrogen (E_2) does not increase, nor does the antiestrogen tamoxifen decrease the growth of Adr^R MCF-7 cells. Moreover, while E_2 induces secretion of specific polypeptides from parental MCF-7 cells and induces progesteron receptors in that cell line, E_2 produce neither effect in Adr^R MCF-7 cells. This lack of hormone sensitivity of MCF-7 cells selected for adriamyecin resistance is associated with a loss of estrogen receptors as measured by hormone binding and antibody precipitation analysis. In contrast, membrane EGF receptors are markedly increased in the Adr^R MCF-7 cells. This increase in EGF receptors is associated with an increase in the responsivenes of the Adr^R MCF-7 cells to EGF (Dr. M. Jett and R. Fine, personal communication) and is also associated with enhanced tumorgenicity in nude mice. While WT MCF-7 cells will form tumors in nude mice only in the presence of exogenic estrogen administration, the multidrug resistant subline readily forms tumors in the absence or presence of estrogen. Thus, this drug resistant cell line is estrogen independent in vitro or in vivo.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06519 05 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Non-Invasive Studies of Metabolism Using Nuclear Magnetic Resonance Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jack S. Cohen, Ph.D.	Research Chemist	MB, COP, DCT, NCI
Gil Navon, Ph.D.	Visiting Scientist	MB, COP, DCT, NCI
Peter Daly, M.D.	Medical Staff Fellow	MB, COP, DCT, NCI
Robbe Lyon, Ph.D.	Senior Staff Fellow	MB, COP, DCT, NCI
Patrick Faustino, M.Sc.	Chemist	MB, COP, DCT, NCI
Peter van Zijl, M.D.	Visiting Associate	MB, COP, DCT, NCI
Gerhard Zugmier, M.D.	Guest Researcher	MB, COP, DCT, NCI
Scott Chesnick	Expert	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Chrit Moonan, BEIB (NMR Center); David Hoult, BEIB; Dennis LeBihan, Diagnostic Radiology, Clinical Center, NIH, Joseph Frank, Diagnostic Radiology, Clinical Center, NIH

LAB/BRANCH

Medicine Branch

SECTION

Biophysical Pharmacology Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We wish to understand differences in metabolism and regulation between normal and cancer cells, and between cancer cells that are responsive to chemotherapy and those that exhibit multiple drug resistance (MDR). Nuclear magnetic resonance (NMR) spectroscopy is used to monitor metabolic processes noninvasively, using a perfusion technique that we have developed in which cells are embedded in gel threads. With this technique, we have observed with ^{31}P NMR significant differences in the levels of major phosphate metabolites in wild type (WT) MCF-7 breast cancer cells, and an adriamycin resistant cell line (AdrR) that exhibits MDR. One of the major differences between normal and cancer cells, is the control of energy metabolism. To follow glycolysis in WT and AdrR cells we use improved the perfusion technique to enable us to use small volumes with ^{13}C-glucose and areable to monitor the rate of glucose uptake and the concomitant rate of lactate production with ^{13}C NMR. The effects of inhibitors on glycolisis have been monitored in the perfusion system. Phospholipid metabolism in these cells has also been monitored by consideration of the levels of phosphomonester (PME) and phosphodiester (PDE) peaks in the ^{31}P NMR spectra. In general, we have found that the higher the PME peak, the more rapidly proliferating are the cells. These results together may provide spectroscopic markers for clinical distinction of growths.

In order to extend these observations to noninvasive metabolic measurements *in vivo*, we have designed and constructed a versatile NMR probe for our spectrometer. All components are adjustable and replaceable, but currently we observe spectra from a subcutaneous tumor in a rodent. We anticipate extending these studies to larger animals and to clinical trials of ^{31}P MRS of breast nodes and limb growths.

CONTROL OF PHOSPHOLIPID SYNTHESIS IN CANCER CELLS:

We have studied the control of phospholipid metabolism in MDA-231 human breast cancer cells by using the agarose gel technique and a new techniques using basement membrane gel threads. We have established that the prominent phosphomo noester peaks in our cell lines are phosphocholine (PC) and phosphoethanolamine (PE) and are intermediates in the synthetic Kennedy pathways and are not degradative products of a phospholipase C (Daly, P.F., et.al., J. Biol. Chem., 262, 14875, 1987). Our data indicate that the rate limiting step of the two pathways are the cytidyl-transferase enzymes and that the level of PC has a rate modulating effect on the rate limiting en zyme. Furthermore, choline and ethanolamine kinase activity is regulated by TGFβ (tumor growth factor-β). Since these enzymes are membrane bound, it has been techni cally difficult to study them by invasive methods since their discovery in the 1950s, and the noninvasive nature of ^{31}P NMR offers a unique opportunity not available by other methods.

Cells were embedded in agarose threads or grown in basement membrane gel threads and then perfused with media or a physiologic salt/glucose solution while being monitored at 37°C by ^{31}P NMR. By adding the substrates ethanolamine and choline to the perfusate we were able to identify the rate limiting step for phosphotidyl-ethanolamine synthesis in this cell line. By use of the choline kinase inhibitors, ethananolamine or hemicholinium-3, we could completely inhibit choline kinases and observe the dependence of phosphocholine cytidylytransferase on the concentration of PC.

The sequential steps choline to PC to cytidine-diphosphocholine (CDP-choline) to phosphoatidylcholine are catalyzed by choline kinase, phosphocholine cytidyltransferase, and cholinephosphotransferase. An analogous three step pathway synthesizes phosphotidylethanolamine. Perfusion with choline caused a rapid and large accumulation of PC but no appearance of CDP-choline. Perfusion with ethanolamine caused a large accumulation of PE but no CDP-ethanolamine. This indicates the rate limiting step in each pathway is the cytidylyltransferase enzyme. It has been controversial (Warden, D.H., Friedkin, M. J. Biol. Chem., 260, 6006, 1985) whether or not the substrate PC is in excess of the Vmax for the cytidylyltransferase enzyme. When choline kinase was completely inhibited by ethanolamine or hemicholium-3 we observed the PC peak to decrease exponentially with a half life of 7 hours while ATP remains unchanged (Daly, et. al.) Since the rate of PC utilization is known to equal the rate of phosphotidylcholine production this indicated that PC has a rate modulating effect on the rate limiting enzyme. The half life of 7 hours compares well to that of 6 hours reported in the literature (Warden, et. al.)

The presence of PE and PC in rapidly proliferating tissue appears to be a marker of increased phospholipid synthesis. We found that MDA-MB-231 cells will grow at the same rate with or without the growth factors in fetal calf serum. TGFβ is known to be an inhibitory growth factor for human breast cancer cells and MDA-MB-231 cells grown in the presence of TGFβ reduced their growth rate by 50% reduction in the amount of PC and PE normalized to βATP. Previous work has shown that choline kinase in fibroblasts is regulated by growth factors (Warden, et. al.) These results indicate that the high concentration of PC and PE found in many tumors are the result of increased choline/ethanolamine kinases activity resulting from the autonomous production of growth factors by cancer cells. This could give tumor cells a growth advantage by increasing the activity of these key synthetic enzymes.

MONITORING THE REGULATION OF PHOSPHOLIPID METABOLISM ASSOCIATED WITH DRUG RESISTANCE:

Phospholipid (PL) metabolism in perfused tumor cells can be monitored by ^{31}P NMR spectroscopy (see above). The two prominent phosphomonoesters (PME) observed phoshocholine (PC) and phosphoethanolamine (PE), are products of choline and ethanolamine kinases, the first steps in PL synthesis of phosphotidylcholine and phosphotidylethanolamine. The primary phosphodiesters (PDE) observed, glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE), are both sub strates for GPC-phosphodiesterase, the last step in PL catabolism.

^{31}P spectra of perfused wild-type (WT) MCF-7 human breast cancer cells are remarkably different from the spectra of an adriamycin resistant (AdrR) subline (Cohen, J.S., Lyon, R.C., Faustino, P.J., et. al, Cancer Res. 46: 4087, 1986.) AdrR cells exhibit lower levels of PME, PDE, and diphosphodiesters (DPDE, primarily UDP-N-acetyl-glucosamine, UDPAG). The effect of choline on the levels of these PL metabolites has been investigated using ^{31}P and ^{13}C spectroscopy.

MCF-7 cells were grown in monolayers in improved minimal Eagle's medium (5% fetal calf serum) containing 14 μM (low) or 400 μM (high) choline under 5% CO_2 environment. Cells were prepared for perfusion by casting in a agarose gel thread matrix as previously described (Foxall, D, Cohen, J.S., Mitchell, J.B., Exp. Cell. Res., 154: 521-529, 1983). Perchloric acid extracts were prepared as described (Lyon, R.C., et.al., Cancer Res., 68: 870, 1986). ^{31}P spectra (162 MHz) and ^{13}C spectra (100 MHz) with ^{1}H decoupling were recorded on a Varian XL-400 spectrometer at 22°C for perfusedor 4°C for extracts. For ^{13}C studies, cells were grown for 72 hours with $^{13}C_{1,2}$-choline enriched media prior to harvest and extraction.
The effect of varying the level of choline in the media during cell growth on the phosphate levels of perfused cells is illustrated in the ^{31}P spectra. High levels of choline greatly enhanced the intracellular concentrations of PC and GPC in the WT cells as predicted, but had no effect on these PL metabolites in AdrR cells. Growth in low choline resulted in the buildup of DPDE levels in WT cells only. The DPDE signals in the WT cell extracts were identified as UDPAG. Growth of cells in ^{13}C choline resulted in specific ^{13}C labeling of PC and GPC, confirming that choline is a substrate for these metabolites. Increasing the ^{13}C choline level from 14 μM to 80 μM enhanced the pool of labeled PC (4x) and GPC (3x) in WT cells and PC (2x) in AdrR cells, but had little effect on GPC in AdrR cells.

In drug-sensitive WT cells, choline regulated PL metabolism by stimulating choline kinase and inhibiting GPC-phosphodiesterase. These enzymes in drug-resistant AdrR cells were much less sensitive to choline levels. The buildup of UPDAG in the WT cells grown in low choline appears to be due to the reduced levels of intracellular PC or its product in PL metabolism, CDP-choline. Both metabolites regulate glycoprotein synthesis by stimulating UDP-acetylglucosaminyl transferase, the enzyme which incorporates UPDAG into glycoproteins (Mookerjea, et, al. B.B.R.C., 36: 851, 1969).

The level of UPDAG in AdrR cells is low regardless of the level of choline or PC. In the absence of glucosamine, UPDAG is synthesized via glucose metabolism The low level of UPDAG in AdrR cells cells may be the result of limited substrate due to the rapid rate of glycolysis (3x higher in AdrR cells) which competes for glucose.

EFFECT OF Ara-C ON PHOSPHOLIPID PATHWAYS:

Ara-C had no effect on ^{31}P spectra of MDA-MB-231 breast cancer cells but caused a pronounced utilization of choline-phosphate and ethanolamine-phosphate in the MOLT4 lymphoma cell line. This utilization is known to be equal to the rate of production of the respective phospholipids since choline-phosphate and ethanolamine-phosphate are the product of an irreversible enzyme, are in a closed pathway, and do not leak from cells. After 8 hours, the ATP and Pi peaks collapsed. This indicates cell lysis, since when cells die without lysing we always see a 3 to 4 fold buildup of Pi.

This result fits very nicely with the phospholipid literature. 1) 10 to 100 micromolar of excess phospholipids have been shown to be lytic to cells. This may explain why cells so tightly couple synthesis and degradation of phospholipids. Although the lytic effects of lysophospholipids are well known, usually the equally potent lytic effect of phospholipids is not observed because they are not soluble and easily transferred through solution. However, under the appropriate conditions excess phospholipids can be inserted into RBC membranes and cause lysis. In our spectra, at least 500 μM of excess phospholipids is being produced. 2) Oleic acid is known to accelerate 5-fold the production of phosphoatidylcholine in HeLa cells without a counterbalancing degradation. This causes a wrinkled appearance and lysis of 60% of the cells within 9 hours. By contrast, phorbol esters cause an increase in both synthesis and degradation without any excess production and no lysis of the cells observed. 3) Studies on leukemia cells from children treated with high dose Ara-C show the intracellular CTP concentration to be 300 μM and the intracellular Ara-CTP concentration to be 400 μM.

It is not surprising that the Ara-CTP would accelerate these pathways since CTP and deoxyCTP are equally effective as cofactors in the phospholipid pathways. Evidently, the hydroxyl group at the 2' position is not needed in these pathways. This makes sense since the intermediates for phospholipid synthesis, cytidine-diphosphcholine(ethanolamine) involve the 5' carbon attaching cytidine by a diphosphodiester bond to choline(ethanoamine). Furthermore, CTP is a key regulatory cofactor in all phospholipid synthetic pathways not just phosphoatidylethanolamine(choline).

An additional point is that the myelin sheaths of the brain contain ample supplies of the enzymes for phospholipid synthesis via the cytidine pathways and *in vivo* NMR studies on humans have shown high concentrations of choline-phsophate and ethanolamine-phsophate in the brain. This may explain some of the cerebrellar toxicity associated with high dose Ara-C.

NMR SPECTROSCOPY IN HUMAN CANCER CELLS PROLIFERATING IN BASEMENT MEMBRANES GEL THREADS:

We have developed a system in which proliferating human breast cancer cells are monitored by NMR spectroscopy for at least six days in basement membrane gel (BMG) threads. The cells are perfused under sterile culture conditions. Cell division in the BMG is easily observed by NMR signal increase while being perfused, by microscope, or by eye as the gel opacifies. Our method is technically simple and requires no adaption of the probe or spectrometer, plus it has the biological advantage of using the natural basement material (Kleinman, et.al., Biochem., 25: 312-318, 1986) for cells which either require or are capable of growing attached to a surface. Normal epithelial or tumor cells grow both on and in this gel and have three dimensional morphologies and functions much more similar to their *in vivo* counterpart than when growth on plastic or glass (Kleinman, et. al.)

Cells are embedded in the gel and then extruded under sterile conditions through 400 μM teflon tubing into petri dishes containing cell culture media and placed in an incubator. The gel is composed of 30% type IV collagen, 60% laminin, 5% nitrogen, 3% heparin sulfate proteoglycan, and 1% entactin; and is commercially available. The cancer cells proliferate in these gel threads as three dimensional spheres and cords. The threads can be pipetted into standard 10mm NMR tubes and perfused. The density in the threads has been measured to be 1 to 2 x 10^8 cells per ml. We were able to maintain sterility by using high volume filters and constructing the cell perfusion system so that it can be easily transported to an autoclave or a sterile bond. The use of filters allows for ease of changing cell culture media or placing additives, as well as obtaining samples for pH and other measurements. Media may be perfused one time only or recirculated.

In MDA-MB-231 human breast cancer cells a signal to noise ratio of ^{15} in ^{31}P spectra can be obtained in 20 minutes with rapid pulsing. Concentrations of the phospholipid precursors phosphocholine and phsophoethanolamine, characteristic of rapidly proliferating tissue (Daly, P.F.,et. al., J. Biol. Chem., 262: 14875, 1978) were increased during logarithmic growth. When perfused with 200 μM hemi-cholinium-3 (a choline kinase inhibitor) the phosphocholine peak disappears after 16 hours while other peaks remain unchanged. UDP-acetylglucosamine and the phospholipid breakdown products glycophosphorylcholine and glycerophosphorylethanolamine, were also observed. The high resolution of this system allows observation of two Pi peaks and indicates the intracellular pH is 6.9 during active growth in pH 7.35 media. This is the same intracellular pH obtained on this cell line grown as solid tumors in nude mice. ^{31}P NMR spectra obtained continuously for up to 64 hours showed and increase in the signals due to increasing number of cells. Spectra of cells in the BMG threads at 20% confluency show a more rapid signal increase than at 60% confluency consistent with Gompertzian tumor growth.

This cell-BMG system is in effect a model tumor, but composed of a homogenous cell population which can be observed indefinitely as the cells reproduce. The high density, high resolution, cellular homogeneity, control of the extracellular environment, and stability of this system will allow prolonged metabolic studies by ^{31}P NMR techniques of phospholipid synthesis and degradation, intracellular pH, glycoprotein synthesis, glycolysis and bioenergentics. This system allows precise control of the extracellular environment and a wide concentration range of drugs, substrates, inhibitors and 13C labeled compoundstoo toxic or too expensive for *in vivo* use. This should aid in the interpretation of *in vivo* spectra currently being obtained from patients.

^{31}P MRS STUDIES *IN VIVO*:

The best model to study human tumors *in vivo* is the arrythymic nude mouse model in which human cancer cells are injected subcutaneously and grow into a tumor. In order to study ^{31}P and other MRS signals of intact tumors *in vivo* in this system we built a versitile NMR probe. In this coil tuned to observe different frequencies is attached to a replaceable copper bracket, thus allowing convenient adaption of the probe to different experiments. Using this system, we have studied the ^{31}P spectra of the same breast cancer cell lines *in vivo* as we studied the *in vitro* perfusion system desribed above.

The results of spectra obtained with the tumors are quite comprable to those in the perfusion system. Two notable differences are that the Pi and the PCr peak are higher *in vivo*. This can be understood by virtue of the presence of much more estracellular space *in vivo* , as well as the presence of subcutaneous muscle. Comparison of the spectra of WT and AdrR cells grown as xenografts also show similar differences to those found in the perfusion system. The major difference between WT andAdrR tumors *in vivo* was the higher level of GPC observed in WT. The loss of GPC during the development of drug resistance may serve as a diagnostic marker. In the MB-231 cells, the PME peaks are high when grown *in vivo* and in the matrigel, indicating a high phospholipid turnover rate. Also, when grown on basement membrane gel an intracellular Pi peak comprable to that *in vivo* tumors was observed. Notably we were not able to disturb the levels of the PME or PDE peaks *in vivo* upon feeding or injecting choline or ethanolamine into the mice, due to processes of excretion and metabolism occuring *in vivo.* This is a case where the controlled conditions achievable in the perfusion system are necessary to shed light on the processes observed *in vivo.*

EFFECT OF ADRIAMYCIN ON ^{31}P SPECTRA OF WILD TYPE AND RESISTANT CELLS *IN VIVO*

The effect of adriamycin on the phosphate metabolites *in vivo* was monitored following 2 different treatment schedules. The first, a single i.v. injection of 12 mg/kg dose, was identical to a treatment reported by Evelhoch, et. al., Cancer Res., 47: 3396, 1987). This allowed for direct comparison of our results with those observed for adriamycin sensitive and resistant murine mammary adenocarcinomas (Evelhoch, et. al., 1987). Tumors are monitored at pretreatment and 1, 2, and 7 days following treatment. A detailed analysis is not yet available since these experiments are still in progress. Preliminary results indicate that the changes are small. This dose of adriamycin is near the LD_{50} at 7 days for these mice. Tumor growth is arrested, but the mice lose about 30% of their body weight within the first 5 days. A less toxic schedule of 3 i.p. injections of 2 mg/kg dose each was given on days 1, 3, 5. ^{31}P spectra were collected on days 0 and 6. Tumor growth was arrested, the mice maintained their body weight and no deaths occured within the first week. No consistent changes in the spectra were observed. These results do not provide a basis for clinical monitoring of adriamycin therapy by *in vivo* ^{31}P spectrospcopy.

^{31}P MRS STUDY OF 2-DEOXYGLUCOSE IN SENSITIVE AND RESISTANT TUMOR CELLS *IN VITRO AND IN VIVO*

Tumors initially responsive to chemotherapy can develop resistance to a variety of antitumor drugs following treatment with only one of them. This phenomena of multidrug resistance (MDR) is one of the primary reasons for treatment failure. We have focused on energy metabolism of wild type (WT) MCF-7 human breast cancer cells and an adriamycin-resistant (AdrR) MDR cell line selected from this parent line. Monitoring glucose utilization and lactate production by ^{13}C MRS and ATP levels by ^{31}P MRS in perfused MCF-7 cells, it was demonstrated that AdrR cells exhibit an enhanced rate of glycolysis and demand for ATP production (Lyon, et. al., Cancer Res. 48, 870, 1988). This difference in energy metabolism suggests that glucose antimetabolites may be more effective in the treatment of drug-resistant compared to non drug-resistant tumors. In this study we have monitored the metabolism of 2-deoxyglucose (2DG), an inhibitor of anaerobic glycolysis, and its effect on energy metabolism in sensitive and resistant tumor cells *in vitro* and *in vivo* by ^{31}P MRS. Also the toxic effects of 2DG on the cell growth in tissue culture was monitored.

MCF-7 cells were grown in monolayers in improved minimal Eagle's medium (IMEM) with 5% fetal calf serum under a 5% CO_2 environment. Cells were prepared for perfusion by casting in an agarose gel thread matrix. Arrthymic nude mice were implanted subcutaneously with MCF-7 cells. The animal probe designed for monitoring tumors *in vivo* was presiously desribed (Lyon, et. al., Magn. Reson. Med., 6:1, 1988.) ^{31}P spectra were collected at 162 MHz onr Initially following the perfusion of AdrR cells with IMEM containing 4 mM glucose and 5 mM 2DG, deoxyglucose-6-phosphate (2DGP, 4.6 ppm) accumulated reducing inorganic phosphate (Pi , 2.4 ppm) and phosphocreatine. After 2DG6P levelled off (2 hr), ATP started to decline slowly restoring the Pi level. WT cells accumulated much less 2GD6P, but ATP levels were exhausted rapidly, followed by a slow decline in glycerolphosphory-choline (GPC, 0.49 ppm). Tumor bearing mice were injected ip with a 2 g/kg dose of 2DG after a 20-hr. fast. For both WT and AdrR tumors, 2DG6P reached a maximum within 1.5 hr. concurrently reducing Pi. No change in ATP or PCr was detected in 3 hr. AdrR tumors accumulated greater levels of 2DG6P. Cells were grown in culture in IMEM containing 11mM glucose and 5 mM 2DG. In 5 days the cell count was reduced to 60% for WT and 33% for AdrR cells.

2DG is a glucose analog which competitively inhibits glucose uptake and glycolytic flux. 2DG was rapidly taken up and phosphorylated by MCF-7 cells. AdrR cells were more receptive for 2DG uptake both *in vitro* and *in vivo*. This enhanced accumulation of 2DG6P may be responsible for the more toxic effects of 2DG on AdrR cells during growth in culture. Both WT and AdrR tumors implanted in mice maintained theirr ATP levels in the the presence of 2DG6P. The differences in energy metabolism observed *in vitro* vs. *in vivo* may be due to a combination of circumstances. Tumor cells *in vivo* have alternative sources of substrate for ATP production. Perfused cells are subjected to a continuous exposure to 2DG. Based on ATP levels, perfused AdrR cells were less sensitive to 2DG6P than WT cells. If the isomerase is inhibited by 2DG6P then the AdrR cells must be utilizing pathways alternative to glycolysis for ATP production. These results suggest that the greater growth inhibition of AdrR cells by 2DG is not due to initial depletion of energy production.

IN VIVO ^{31}P NMR SPECTROSCOPY APPLICATION TO PHOSPHORUS METABOLITES IN MUSCLE

The *in vitro* measurement of diffusion by NMR is a well established technique. Recently, it has been shown that *in vivo* diffusion of water can be studied by Magnetic Resonance Imaging (Le Bihan, D., et.al., Radiology, 161: 401, 1986). Using similar techniques, the diffusion coefficients of individual metabolites may be studied *in vivo* by diffusion spectroscopy. The purpose of this work is to demonstrate the feasibility of *in vivo* diffusion measurements of cellular metabolites using diffusion spectroscopy, and to apply this technique for the demonstration of restricted diffusion of phosphocreatine in rat muscle tissue using ^{31}P NMR.

The Stejskal-Tanner (Stejskal, E.O. and Tanner, J.E., J. Chem. Phys. 42: 288, 1965) spin-echo pulse sequence was slightly modified for the *in vivo* diffusion spectroscopy and implemented on the 2T GE CSI instrument with shielded gradient coils. The sequnce and gradient performance were designed to minimize eddy current effects. At least four measurements were carried out using different values of the gradient strength G (up to 15 G/cm). Then, the diffusion constant D was determined. A six-turn solenoid coil of the size of a rat leg was used. Measurements were performed on phantom samples of phosphocreatine in water (20mM) and on the upper hind leg of the Sprague-Dawley rats.

Following the established theory (Cooper, R.L., et.al., Biophys. J. 14: 161, 1974), the calculated diffusion coefficient should be regarded as an apparent diffusion coefficient D^a because of the possibility of restricted motion due to the presence of boundaries or obstacles to diffusion. In order to test these effects, the apparent diffusion coefficient D^a plotted as a function of the diffusion time. The decrease in D^a upon increasing diffusion time clearly indicates the influence of the restricted diffusion. Since the diffusion coeeficient of free phosphocreatine in muscle tissue will be smaller or equal to that of free PCr in water at 37°C the data allow an approximation of the size of the restricted volume. The simulation leads to a size of 13 μM for the restricted volume This size fits well with the known dimensions of a normal muscle cell.

The results clearly demonstrate the feasibility of diffusion spectroscopy *in vivo*. The noninvasive measurement of the apparent diffusion constant of individual metabolites should be a useful tool for measuring the apparent compartment size and the (im)mobilization of metabolites.

INSTRUMENTAL IMPROVEMENTS

The G.E. Signa 1.5T body imaging system has been modified to accept a fuse based safety device to prevent injury due to F. Power faults. The G.E. surface coils have been modified to improve sensitivity regardless of patient loading.

Double timed and straddle suface coils have been designed ($^{31}P/H^1$, Na/H^1, H^1/H^1). At present these are under construction in the BEIB machine shop. Additional limb and localized coils for patient use are under design (shoulder, neck, lumbar, and knee). A variety of specialized materials for these have been acquired.

The 4.7T CSI system has been upgraded to accept the self-shielding gradients. These are working and have elimated the distortions due to gradient eddy currents. The new coils allow high resolution imaging and localized spectroscopy. The gradient set is easily removed to allow large animal measurments (about 5 minutes). A new millidegree phase-shifter is being installed on the 4.7 T system, and an ethernet connection allowing file tansfer to a sun workstation for data analysis.

The following coils have been built for the 4.7T system:

Surface coils

- 20mm C^{13}/40mm H^1 coaxial
- 40mm C^{13}/H^1 double tuned
- 40mm H^1
- 40mm P^{31}/Na coaxial
- 80mm H^1
- 15mm F^{19}

High Resolution

- 20mm P^{31} solenoid coil high resolution
- 20mm H^1 solenoid coil high resolution

Tumor coil for rodents with gas isolation chamber

- H^1 25mm
- P^{31} 25mm
- P^{31}/H^1 (under construction)

Imaging Coils

Na/H^1 50mm x 20cm saddle type (2T)
H^1 50mm x 20cm saddle type

The prototype birdcage coils have been built for evaluation at 200 MHz. These were diassembled for their component capacitors when they gave unsatisfactory results.

Work continues on 75mm and 50mm diameter birdcage coils for use with shielded gradients. Design of double tuned birdcage coils has been explored and one one prototype dual mode resonator has been built.

Four protoype anterior neck coils have been built and evaluated for the Picker (0.5 T) system. This experience will be of value for applications to the Signa system. This project is still in progress. The prototype has given better resolution than the existing Picker system. Problems with the remote tuning system have been preventing optimum sensitivity.

Projects under construction or in the design phase include:

1) Rat probe for the 300 MHz Bruker system
2) Remote tuning capability
3) Double tuned single port surface coils
4) R.F. shielding for CSI system
5) Cable bulkhead for the CSI magnets
6) Low noise preamps for CSI

Localized spectroscopy programs have been started. The G.E. stimulated echo sequence has been improved by adding crushers to obtain a clean signal of the voxel of interest. Programs that are now working include stimulated echo (STEAM) and point resolved spectroscopy (PRESS). Shimming on the voxel of interest gave line width of about 1Hz for water in a phantom (5x5x5mm) and a line width of 8 Hz in a rat head of the same volume. Other investigators never achieved better than about 25-50 Hz. At present water-suppression is being built in.

Preliminary experiments on protons show a strong influence of susceptibility differences on the techniques used. High gradient values are necessary to ensures that the volume of interest selected is really obtained. The stimulated echo has also been tested for ^{31}P and works well, although of course s/n is quite low for these small volumes.

A set of high pass band pass and band rejection filters have built to do hetero-CSI-nuclear decoupling exp. on the 4.7T system. The stimulated echo program on the Signa has been improved (Chrit Moonan and Dennis Le Bihan) and works now. Water suppression will also be installed.

PUBLICATIONS:

Lyon, R.C., Cohen, J.S., Faustino, P.J., Megnin, F. and Myers, C.E.: Glucose Metabolism in Drug-Sensitive and Drug-Resistant Human Breast Cancer Cells Monitored by Magnetic Resonance Spectroscopy. Cancer Res., 68: 870, 1988.

Daly, P.F., Lyon, R.C., Faustino, P.J. and Cohen, J.S.: Phospholipid Metabolism in Cancer Cells Monitored by ^{31}P NMR Spectroscopy. J. Biol. Chem. 262: 14875, 1988.

Cohen, J.S. and Lyon, R.C.: Multinuclear NMR Study of the Metabolism of Drug-Sensitive and Drug-Resistant Human Breast Cancer Cells. N.Y. Acad. Sci., 508: 216, 1987.

Lyon, R.C., Tschudin, R.G., Daly, P.F. and Cohen, J.S.: A Versatile Multinuclear Probe Designed for In Vivo Spectrospcopy: Applications to Subcutaneous Human Tumors in Mice. Mag. Res. Med. 6:1, 1988.

Daly, P.F., Lyon, R.C., Straka, E.J. and Cohen, J.S.: ^{31}P NMR Spectroscopy of Human Cancer Cells Proliferating in a Basement Membrane Gel. FASEB J., in press.

Lyon, R.C., Daly, P.F. and Cohen, J.S.: Effects on the Metabolism of Human Cancer Cells In Vitro Implanted in Mice Monitored by Magnetic Resonance Spectroscopy. In *Benzon Fndtn, Symp.* No. 26 Copenhagen. "NMR Spectroscopy in Drug Development" (Jaroszewski, J.W., Schaumburg, K. and Kofod, M., eds.), in press, 1988.

Cohen, J.S.: Phospholipid and Energy Metabolism of Cancer Cells Monitored by ^{31}P Magnetic Resonance Spectroscopy: Possible Clinical Significance. Mayo Clinic Proc., in press.

Daly, P.and Cohen, J.S.: Magnetic Resonance Spectroscopy of Tumors and Potential In Vivo Clinical Applications: A Review. Can. Res., in press.

Cohen, J.S., Lyon, R.C. and Daly, P.F.: Monitoring Intracellular Metabolism by NMR Methods In Enzymology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06520-05 CP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Contrast Agents for Magnetic Resonance Imaging of Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jack S. Cohen, Ph.D.	Research Chemist	MB, COP, DCT, NCI
Patrick Faustino, M.Sc.	Chemist	MB, COP, DCT, NCI
David Place, Ph.D.	Guest Researcher	MB, COP, DCT, NCI
Peter van Zijl, M.D.	Visiting Associate	MB, COP, DCT, NCI

COOPERATING UNITS (if any)
Institute for Diagnostic Research, Berlin, West Germany

Medicine Branch

LAB/BRANCH
Biophysical Pharmacology Section

SECTION
NIH, National Cancer Institute, Bethesda, MD 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Magnetic resonance imaging (MRI) is an important tool in diagnostic radiology, and allows discrimination between a growth and surrounding soft tissue. The contrast depends of the relaxation times (T_1 and T_2) of the bulk water in the respective tissues. A paramagentic substance is one that contains an unpaired electron and alters the relaxation rate of water. Our objective is to identify a class of paramagnetic contrast agents which are selectively retained by tumors, in order to facilitate and clarify MRI tumor contrast.

We have chosen the water-soluble metalloporphyrins (WSMPs) for several reasons: porphyrins are known to be selectively retained by tumors, they form complexes with metal ions, and the synthetic water soluble porphyrins are much more tractable than the hydrophobic natural heme products. We have evaluated several of these WSMPs by several criteria: solubility; relaxivity; stability in human plasma and in vivo; distribution in vivo; toxicity; and MRI contrast properties. As a result of these comparisons we have identified Mn(III) TPPS as a potential tumor contrast agent in MRI. We are currently studying the mechanism of tumor retention and stability of porphyrins and WSMPs, and quantiative dose-contrast relationships in MRI of nude mice and rat tumors for a series of WSMPs.

Manganese-Porphyrin MRI Contrast Agents for Tumors

We had previously reported that the water soluble manganese complex of tetra(4-phenylsulfonato)porphyrin, Mn(III)TPPS is a stable and effective contrast agent for tumor detection by magnetic resonance imaging at 0.5 Tesla (Patronas, et.al., Cancer Treatment Reports, 70: 391, 1986). To address the issue of *in vivo* stability and tissue concentration, analytical instrumentation in our laboratory has been upgraded to allow the determination of trace quantities of metal complex and metal-free porphyrin. A number of other analytical biochemistry techniques are being employed to determine the mechanism by which Mn(III)TPPS binds to cells, and whether it accumulates inside cells or on the surface of the cell membrane. With the recent installation of two small animal imaging units at the NIH *in vivo* NMR Research Center, our ability to obtain high quality imaging data has been greatly enhanced. In our recent studies, nude mice bearing subcutaneous implants of LS-174T human colon carcinoma or MCF-7 breast carcinoma were injected with MnTPPS and imaged at 2.0 Tesla on successive days after injection (van Zijl, et. al., Acta Radiologica, in press). Although the bulk of the injected dose had been excreted within a day, even after 8 days the tumor itself still exhibited enhanced contrast due to the retained agent. Future imaging experiments include studies of dose dependence on observed contrast in both nude mice and in rats. In addition, a series of new porphyrin complexes with similar chemical properties to MnTPPS are being synthesized. These agents will be tested for their stability, tumor uptake and retention, and pharmacological behavior.

PUBLICATIONS

Megnin, F., Faustino, P., Lyon, R.C. and Cohen, J.S.: On the Mechanism of Porphyrin and Metalloporphoryin Uptake in Cancer Cells, Biochem. Biophys. Acta., 929: 173, 1987.

Van Zijl, P., Place, D., Cohen, J.S., Faustino, P.J., Lyon, R.C., Patronas, N.J., Metallopophyrins Magnetic Resonance Contrast Agents: Feasability of Tumor-Specific MRI, Acta Radiologica, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06521-05 CP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conformations and Interactions of Nucleic Acids, Proteins and Drugs in Solution

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jack S. Cohen, Ph.D.	Research Chemist	MB, COP, DCT, NCI
To Be Appointed	Biotechnology Fellow	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Medicine Branch

SECTION
Biophysical Pharmacology Section

INSTITUTE AND LOCATION
NIH, National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We wish to elucidate conformations of DNA in solution, and to investigate their relationships to genetic function, interactions with drugs, and protein recognition. The method we have chosen to carry out such studies is nuclear magnetic resonance (NMR) spectroscopy; more specifically the method used to detail these solution conformations is the 2D-NOE NMR method, which provides relative interatomic distances.

Loops in DNA are expected to be important for protein recognition of selective genetic sites, such as RNA polymerase promotor sequences. Consequently, we have studied quasipalindromic synthetic oligonucleotides and the mechanism of hairpin loop formation.

In addition to studies with normal oligos, we are investigating phosphorothioate oligo duplexes by the above NMR methods. This is complicated by the fact that due to non-sterospecific synthesis each thio-phosphate can exist in two sterospecific forms. NMR should help to clarify the conformation(s) of the low temperature duplex form of oligo analogs.

Conformation of an Oligo with an Extra A

The conformation of an oligodeoxynucleotide pentadecamer d(CGCGAAATTT-ACGCG), self-complementary except for an additional adenosine nucleotide at position 11, has been investigated with NMR spectroscopy. This oligomer was found to exist predominantly in the duplex form in solution, rather than in the hairpin loop form as observed previously for an analogous 13-mer sequence d(CGCGAATTACGCG). Nuclear Overhauser effects indicate that the B-form conformation is disrupted by the extra A base, which forms a wedge within the duplex, producing a bent junction and an overall 'S' like structure. A down-field shifted phosphate resonance was assigned using a two-dimensional ^{1}H-^{31}P correlation method, and found to be P12-13. Interestingly, an X-ray crystal structure of the same pentadecamer showed the extra A outside the helix in two distinct structures (Miller, et.al., personal communication). This may arise from the very strong packing forces that are present in the crystal, causing the helices to lie parallel and retain the linear B-form.

Molecular Graphies and Conformational Calculations

During the past year an Evans & Sutherland PS390 molecular graphics system was purchased and interfacedwith a DEC micro-VAX-II mini computer. Two display programs, MOGLI and FRODO have been set-up and several molecular structures have been tested. The energy minimization-molecular dynamics progam AMBER has been set-up, but not yet tested. A program, which enables distance calculations from 2DNOE data, has been implemented, but cannot yet be used. It is intended to use this system to investigate the conformations of groups linked to DNA duplexes, and to calculate structures derived from 2DNOE data.

PUBLICATIONS

Roy, S., Skenar, V., Appella, E. and Cohen, J.S.: Conformational Pertubation Due to Extra Adenosine in a Self-Complementary Oligodeoxynucleotide Duplex. Biopolymers, 26: 2041, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06523 04 CP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism, Irreversible Binding and Mechanism of Action of Etoposide (VP-16,213) to Cellular Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Birandra K. Sinha, Ph.D.	Pharmacologist	CPB, COP, DCT, NC
Pedro Politi, M.D.	Visiting Fellow	CPB, COP, DCT, NCI
Lata Dusre, Ph.D.	Visiting Fellow	CPB, COP, DCT, NCI
Helen M.Eliot	Biologist	CPB, COP, DCT, NCI

COOPERATING UNITS *(if any)*
Dr. Y. Pommier, NCI, NIH; Dr. B. Kalyanaraman, Medical College of Wisconsin, Miwaukee, WI.

LAB/BRANCH Clinical Pharmacology Branch

SECTION Biochemical Pharmacology Section

INSTITUTE AND LOCATION
NIH, National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

VP-16 undergoes O-demethylation to generate active intermediates that binds to protein and DNA. The O-demethylation is P450 dependant. Peroxidases, such as horseradish or prostaglandin synthetase, also activate VP-16 and VM-26 to their O-Quinonederivatives, and catalyze binding of reactive inermediates to DNA. Enzymatic activation to reactive intermediates may be important in the biological activities of VP-16 and VM-26.

The semisynthetic podopyllotoxin derivative etoposide (VP-16) has shown activity against a number of human tumors. Although the mechanism of action of this drug is not clear, DNA damage induced by VP-16 has been suggested for its cytotoxicity. Recently, we have proposed that the cellular damage induced by VP-16 may result from the formation of a reactive immediate during bioactivation of the drug. We have studied the metabolism of VP-16 by mouse hepatic microsomes. Using HPLC analysis of the chloroform extracts of the microsomal incubation it was shown that VP-16 formed the 3'-4'dihydroxyl derivative (DHVP). The formation of this metabolite (2% of the parent drug) was NADPH-protein-VP-16 and time dependent suggesting that the activation was enzymatic. Moreover, DHVP formation was inhibited by SKF-525A and piperonylbutoxide suggesting that the 0-demethylation was P-450 dependent. Incubation of [^{3}H] VP-16 with microsomes containing NADPH and DNA resulted in irreversible binding of the drug to DNA and proteins.

Recently, we have found that peroxidase catalyzed activation of VP-16 forms a number of reactive metabolites. HPLC and mass spectral analysis have shown that VP-16 undergoes aromatization (to Ar-VP-16-Q) which is subsequently 0-demethylated to 0-Quinone (Ar-VP-16-Q). Inhibition studies suggest that the protein binding species result from 0-demethylation reactions (VP-16-Q and AR-VP-16-Q) and that DNA binding species are positively charged.

Using alkaline elution studies in a sensitive and resistant MCF-7 cells recently, we have found that VP-16 induces a significant amount of DNA damage in the sensitive cells. In contrast, very little DNA damage could be detected in the resistant cells. Furthermore, when isolated nuclei were used to assess DNA damage, there was only two-fold difference in VP-16 induced DNA strand breaks between the sensitive and resistant cells. The differences in toxicity (~200 fold), and uptake of VP-16 (2-3-fold) do not completely explain DNA damage induced by VP-16 in these cells and suggest that other factors may also be involved in the mechanics of cell kill by VP-16.

Resistance to VP-16 and other antitumor drugs results in a decreased drug accumulation and in multidrug resistant cell lines overexpression of P-170 glycoprotein has been implicated in this decreased drug accumulation. In order to characterize VP-16 resistance, we have carried out uptake and efflux of VP-16 in a number of sensitive and resistant human tumor cell lines. Our studies suggest that decreased VP-16 accumulation is not due to overexpression of the P-170 glycoprotein, but it may be related to energy-dependent modification in drug binding in the resistant cell lines.

Recent studies also indicate that a VP-16 metabolite, dihydroxy VP-16, formed from 0-demethylation of VP-16 chelates metal ions and in the presence of H_2O_2 or reduced gluatathione forms hydroxyl radicals which induce DNA cleavage.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06524 02 CP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Inhibition of Gene Expression by Oligodeoxynucleotide Analogs

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Normal oligodeoxynucleotides (ODNs) have been reported to have inhibitory effects on selected gene expression, primarily due to transcription arrest by mRNA duplex formation. However, these compounds are susceptibile to digestion by cell nucleases. Nuclease-resistant ODN analogues containing methylphosphonate (P-Me) in place of phosphate has been developed to overcome this problem, but these compounds also have disadvantages, notably poor aqueous solubility and poor hybridizability. In order to overcome these difficulties, we have synthesized a series of phosphorothioate (P-S) analogs, which retain the charge of phosphate, but are nuclease-resistant. These compounds are being tested as agents against HIV and other lentiviruses, against herpes simplex Virus (a DNA virus), and several oncogenes (eg. myc) in lung cancer cells, and against genes related to drug resistance (eg. P-170 genes). Tests for toxicity in mice are also being conducted. A series of analogs with covalently attached groups on both the 3' and 5' ends of oligomers are being synthesized and will also be tested for biological activity in a cell free expression system, and in the systems tested above if further evidence of improved activity is discovered.

MAJOR FINDINGS

Anti-HIV Studies:

In previous work, we showed that the homopolymer S-dC_{28} was the most effective oligo for inhibiting the cytotoxic effects of HIV. In addition to this non sequence-specific antiviral activity of phosphorothioate oligomer against de novo infection of HIV in uninfected T-cells, we have investigated the sequence specific antiviral activity of phosphorothioate oligomer to inhibit viral expression of HIV in chronically infected T-cells. Phosphorothioate oligomer (28-mer) with antisense sequence against the art/trs initiation site of HIV has significant, selective inhibitory effect on p24 gag protein production and gp120 env protein in chronically HIV-infected-T-cells. However, none of the phosphorothioate oligomer control sequences tested (sense, random, N-methyl-thymidine-containing antisense sequence, or homo-oligomer nor unmodified normal oligomer with antisense sequence) had any inhibitory effect on viral expression. Remarkable changes observed in the HIV mRNA profile with the antisense phosphorothioate oligomer against art/trs suggests that the mechanism for inhibition of viral expression is due to interfernce in art/trs encoded protein synthesis by translation arrest mechanism. This unique property, regulatory activity against viral gene expression, could give phosphorothiote oligo analogues great potential for antiviral treatment against AIDS and related disorders.

Physiochemical Properties of Phosphorothioate Oligos:

Because certain phosphorothioate oligodeoxynucleotide (S-oligo) analogs, unlike their normal congeners, have been found to exhibit siginificant anti-HIV activity we have studied their physiochemical properties. We have measured themelting temperatures (Tm) of a series of S-oligos compared with those of the corresponding normal oligomers. The Tm's for AT base pairs of S-oligos are signifcantly depressed relative to normal oligos, while GC-containing S-oligos show much less Tm depression.

The Tm's of S-dT oligomers with poly-rA are reduced relative to the duplexes with normal dA oligomers. These results provide a rational basis for the S-d(CG) sequences as anti-message inhibitors of gene expression. We also developed an automated synthesis of 5'-acridine linked oligothymidylates using phosphoramidite-linked acridine. During this synthesis we noted the replacement of thiophenol for the 6-chloro substituent on acridine. We have measured the Tm's of the compounds with 3 and 5 methylene groups linked to normal and phosphorothioate dT_n (with n=3-40) on duplex formation with the equivalent dA_n, and have found small increases of Tm for the 5-methylene linked acridine derivative. We have monitored the uptake of these fluorescently labelled oligos into HL60 cells. and found that the shorter oligos sre more rapidly taken up than the longer, and the normal oligos faster than the S-oligos. The temperature dependence of the cellular uptake suggests an energy dependent process, and a possible membrane receptor for oligos. These results have significance for the potential use of such compounds as inhibitors of gene expression.

Physiochemical Properties and Synthesis of Phosphoselenate Analogues of Oligodeoxynucleotides:

Because the phosphorothioate analogues of oligos have been found to exhibit anti-HIV activity, we have synthesized and characterized the phoshoselenate analogues. These has also been found to have anti-HIV activity although not greater than the P-S analogue. We are characterizing these analogues further, but in view of the the lower stability of the P-Se compound to the P-S compounds we do not expect them to have significant clinical usage.

Synthesis and Properties of Novel 5'-Linked Oligos:

We have synthesized oligos with 5'-linked acridines in the automated synthesizer using an intermediate acridine-linked phosphoramidite. With the acridine moiety linked via the 9-amino position, 6-chloro-2-methoxyacridine resulted in facile substitution by thiophenol at the 6 position. Using a non-substituted acridine gave 5'-linked dT oligos that exhibited significant Tm increase in duplexes with dA, as observed previously by Helene and co-workers. However, use of blocked A,G, and C monomers resulted in base-catalyzed cleavage of the linker at the 9-amino acridine position. In order to overcome this problem, we used an alternative synthesis with a maleimide linked acridine (Nara & Tuzimura, Agric. Biol. Chem., 42: 793, 1978), which was attached to a mercaptopropanol linker (Connolly, Nucl. Acids Res., 13: 4485, 1985). In this way we were able to prepare linked acridine with any sequence oligo. We have also prepared anthroquinone-linked oligomer using a linked phosphoamidite that is not sensitive to alkaline cleavage.

Uptake of Oligos into Cells:

When small antisense oligodeoxynucleotides (ODNs) complementary to the 5' region of *c-myc* mRNA are added to intact cells in culture, *c-myc* protein synthesis is specifically inhibited. Furthermore, addition of specific antisense ODNs to culture medium inhibits intracellular viral replication. These data suggest that unsubstituted ODNs can pentrate cells. However, such findings contrast with the prevailing view that cells are impermeable to ODNs. In order to design more effective synthetic antisense ODNs as potential clinical agents, we synthesized oligo dT_n compounds containing 5'-linked acridine and utilized these fluorescent derivatives, in conjunction with flow cytometric analysis, to examine the mechanism of cellular uptake. Wefound that these compounds, up to 20 bases in length, are taken up by cells in a saturable and size-dependent manner compatible with a receptor mediated endocytotic process. Although neither nucleosides nor deoxyribose 5' phosphate are comptetitors of uptake, polynucleotides of any length will competitively inhibit ODN transport, providing they possess a 5' phosphate moiety. Using oligo dT-cellulose as a tool for affinity purification, we have identified an 80 kD cell surface protein which may mediate ODN transport.

Phosphorothioate Oligodeoxynucleotides are Non-Sequence Specific Inhibitors of Translation in Cell-Free Systems:

Phosphorothioate oligodeoxynucleotides (S-ODNs) have recently been shown to inhibit the cytopathic effect of HIV 1 by an antisense mechanism (Matsukura, M., et. al., Proc. Natl. Acad. Sci. USA, 84: 7706, 1987). In HL-60 cells, however, an antisense *c-myc* phosphorothioate construct failed to consistently inhibit cellular proliferation, while the all-oxygen congener was effective (Loke, S.L., et. al., Curr. Topics. Microbiol. Immul., in press). We studied the effect of S-ODNs on the translation of rabbit globin mRNA in the rabbit for reticulocyte lysate and wheat germ cell free translation systems. S-ODNs were synthesized and purified as previously described (Stein, C., et. al., Nucl. Acids Res., 16: 3209, 1988), and all oligomers were synthesized on an ABI Model 380B Synthesizer. mRNA was translated into protein in the presence of 35S methionine, loaded onto 15% SDS-PAGE gels (19:1 and 37:1 acrylamide/bisacrylamide, reticulocyte lysate and wheat germ systems respectively), and subjected to electrophoresis. Gels were then fixed, dried and autoradiographed.

A 15-mer complementary to the α-globin initiation codon and downstream region (ODN 1) was initially studied. With the all-oxygen compound, concentration dependent inhibit of globin synthesis was observed (100% $\alpha+\beta$ at 100 μM), while no inhibition was seen for the sense analogue. Both the sense and the antisense phosphorothioate 15-mers were 100% inhibitory ($\alpha+\beta$ globin) above 6.25 μM, while control levels were reached at 1.6-3.1 μM.

The effect of phosphorothioate ODN length was also evaluated: A random 5-mer S-ODN was not inhibitory at any concentration (up to 100 μM), while a random 10-mer was 100% inhibitory at 100μM. Control levels were observed at 25 μM. The homocopolymer S-dC28 behaved similar to ODN 1, while its oxygen analog was not inhibitory up to 100 μM.

The non-sequence specific effect of S-ODNs was further demonstrated in the wheat germ system. The sense construct of ODN 1 was 100% inhibitory to translation of Brome Mosaic Virus mRNA at 12.5 μM. As in the lysate system, the random 5-mer was not inhibitory (up to 100μM), but with the 10-mer, control levels were not reached until 3.1 μM. With S-dC28, 90% inhibition was still observed at 125 nM. The marked oligomer length effect rules out metal-ion binding and synthesis impurities as causes of protein translation inhibition. Efforts aimed at determining the specific targets of phosphorothioate ODNs in these systems are currently under way. For this purpose, we have synthsized ^{35}S-dC$_{28}$ radiolabelled oligomer via the M-phosphorate method.

Inhibition of *c-myc* Expression in HL60 Cells:

The effects of addition of 50 µM PO-and PS-ODNs to log phase HL50 cells was observed. While intracellular *c-myc* protein levels fall within 4 hours of PO-ODN addition, reaching a level approximately 30% of control within 18 hours, addition of PS-ODN was ineffective in lowering intracellular *c-myc* protein. We thought that PS-ODNs might have difficulty in entering the cells, since their overall stability is much greater than that of PO-ODNs. This was confirmed by monitored the uptake of acridine labelled PO-and PS-ODNs by cytometry (see above).

While uptake of PO-ODN (oligo-dT_7) was clearly observable within 4 hours and plateaued at 40-50 hours, the uptake of an identically sized PS-ODN was barely detectable, even with 50 hours of incubation. To novercome this difficulty and utilize the stability of the PS-ODNs, we loaded them in liposomes and fused these vesicles with the cells. The effects on *c-myc* protein expression were seen. Both PO- and PS-ODNs antisense to *c-myc* mRNA are effective in reducing *c-myc* protein expression within 1.5-2 hours following lipofusion. Empty liposomes and those containing nonsense sequences are ineffective. The inhibition of *c-myc* protein expression is transient and is nearly completely reversed within 24 hours of lipofusion. Nonetheless, both cell growth and DNA synthesis are inhibited specifically by lipofusion with PS-ODNs antisense to *c-myc* mRNA. The inhibition of DNA synthesis is reversible and follows a similar time course to that of *c-myc* protein expression following lipofusion. The fact that growth is nearly completely inhibited for 24 hours suggests that the effects of PS-ODN antisense to *c-myc* persist for nearly that time period.

HIV Reverse Transcriptase: Effects of Phosphorothioate Oligos:

The inhibition of the polymerase activity of HIV-RT has been studied in the presence of increasing concentrations of S-oligos. Compared to normal controls, the S-oligo exhibited strongly competitive inhibition (as measured by reduced uptake of tritiated thymidine). The ratio of K_I compared to normal oligo was ca. 10^3. Comparison of different lengths indicated that the longer sequences were more effective, but no sequence-dependence was found. A series of block co-polymers in which nine of the 27 phosphates in dC_{28} were substituted with PS in the 3' and 5' end and middle, all showed approximately the same degree of inhibition midway between that of normal and all-PS oligo. These results indicate non-selective binding of PS groups to the primer binding site of RT. A filter binding assay with S-dC_{28} also revealed significantly enhanced binding to RT, with one strong binding site with $K_d \approx 10^3$ x that of normal oligo, and several weaker binding sites. These results may have relevance to the mechanism of inhibition of the cytotoxic effect of HIV inhibited by S-oligos.

Comparative studies are underway on the inhibition of several human polymerases by the S-oligos.

Inhibition of Lenti- and other Viruses:

In view of the inhibitory effect of S-oligos on HIV, studies on their effects on other viruses have been carried out. Very similar results, namely non-sequence specific inhibition by S-dC_{28}, was observed for several animal lentiviruses (CAEV, EIAV). However, no comparable inihibtion was observed with the retrovirus is not a lentivirus of the same family as HIV. This may relate to differences in thebinding to the reverse transcriptases which are different and have different metal ion content. However, the reasons for this difference is unclear at present. Studies with other viruses, including herpes simplex virus are well underway.

PUBLICATIONS

Matsukura, M., Shinozuka, K., Zon, G., Mitsuya, H., Reitz, M., Cohen, J.S. and Broder S.: Phosphorothioate Analogs of Oligodeoxynucleotides: Novel Inhibitors of Replication and Cytopathic Effects of Human Immunodeficiency Virus (HIV), Proc. Natl. Acad. Sci., USA, 84: 7706, 1987.

Stein, C.A., Subasinghe, C., Shinozuka, K. and Cohen, J.S.: Physiochemical Properties of Phosphorothioate Oligodeoxynucleotides, Nucl. Acids Res., 16: 3209, 1988.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06709-06 M

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October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Drug Resistance

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	Karen Grotzinger	Med Technologist	M	NCI
	Thomas C. Hamilton	Sr. Staff Fellow	M	NCI
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Medicine Branch

SECTION

Experimental Therapeutics

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS 6	PROFESSIONAL 5	OTHER 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the biology of ovarian cancer, the mechanisms of antineoplastic drug resistance in ovarian cancer, and the pharmacologic reversal of the drug resistance. These studies have been aided by the development of unique model systems of ovarian cancer including 10 new ovarian cancer cell lines and an intraperitoneal model of human ovarian cancer with ascites, pulmonary metastasis, and death from intraabdominal carcinomatosis. In addition, we have developed drug resistant variant cell lines which are 6-100 times more resistant to chemotherapy than the sensitive parental cell line from which they were derived. Using these model systems we have demonstrated that resistance to melphalan and cisplatin is linked to elevations in cellular glutathione levels. We have also shown that buthionine sulfoximine (BSO), a synthetic amino acid which inhibits synthesis of glutathione, leads to a decrease in glutatione levels in vitro and in vivo and increases the cytotoxicity of melphalan. In addition, we have demonstrated that drug resistance to cisplatin in these model systems of ovarian cancer is also related to increased capacity for the cells to repair DNA damage. Aphidicolin, an inhibitor of the enzyme DNA polymerase increases the cytotoxicity of cisplatin in the resistant cell lines by blocking DNA repair. In addition, glutathione also facilitates the repair of platinum and DNA damage. The combination of aphidicolin and BSO leads to maximum depletion of repair capacity. The molecular basis for cisplatin resistance is being investigated with the goal of identifying and cloning the responsible gene. It has also been demonstrated that some human ovarian cancer cell lines have increased amplification/expression of the mdr-1 gene although all the cell lines examined thus far do not have any decrease in drug accumulation of

natural products. Consequently, the role of *mdr*-1 expression of drug resistance in ovarian cancer remains to be determined.

Ongoing Studies:

1. *Model Systems of Human Ovarian Cancer*. We have established 10 long-term cell lines of human ovarian cancer. In vitro drug resistant variant cell lines have been established from drug sensitive cell lines by exposure to antineoplastic agents. The combination of in vitro and in vivo selection procedures was used to establish a transplantable human ovarian cancer in nude mice which produces ascites and intraabdominal carcinomatosis. Other cell lines are also being adapted for subcutaneous intraabdominal growth in nude mice.

2. *Mechanisms Of Drug Resistance*. We are focusing our studies on the mechanisms of resistance to cisplatin and melphalan. Studies are in progress to study the role of GSH in the formation of DNA-cisplatin adducts, and in the repair of DNA damage in drug resistant cell lines. The specific enzyme systems involved in DNA repair and the specific lesion responsible for cisplatin cytotoxicity is being investigated. In addition, the role of gene amplification and expression of DNA repair enzymes is being evaluated. Studies upon the importance of metallothionein in cisplatin resistance are also underway.

3. *Alterations Of Drug Resistance*. We are continuing to examine the role of potential modifiers of drug resistance in human ovarian cancer cell lines. in vitro and in vivo. Manipulations of glutathione alter sensitivity to melphalan and cisplatin. We have demonstrated that buthionine sulfoximine plus melphalan prolongs survival in the nude mouse model compared to treatment with melphalan alone providing direct evidence that GSH modulation may be clinically feasible. A clinical protocol is being developed to examine buthionine sulfoximine plus melphalan in drug resistant patients.

5. *Immunotherapy of Ovarian Cancer*. In collaboration with Dr. Ira Pastan, Laboratory of Molecular Biology, DCBD we have demonstrated that monoclonal antibodies linked to toxins (pseudomonas, ricin) are cytotoxic in vitro and in vivo to human ovarian cancer cells. Clinical protocols have been initiated based on these observations.

PUBLICATIONS

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2. Louie, K.G., Behrens, B.C., Kinsella, J.J., Hamilton, T.C., Grotzinger, K.R., McKoy, W.M., Winker, M.A., and Ozols, R.F.: Radiation survival parameters of antineoplastic drug-sensitive and resistant human ovarian cancer cell lines and their modification by buthionine sulfoximine. *Cancer Res.* 45: 2110-2115, 1985.

3. Reed, E., Yuspa, S.H., Zwelling, L.A., Ozols, R.F., Poirier, M.C.: Quantitation of cisplatin-DNA-intrastrand adducts in testicular and ovarian cancer patients receiving cisplatin chemotherapy. *J. Clin. Invest.* 77: 545-550, 1986.

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6. Louie, K.G., Hamilton, T.C., Behrens, B.C., Grotzinger, K.R., McKoy, W.M., Myers, C.E., Young, R.C., and Ozols, R.F.: Intraperitoneal Aclacinomycin A for the treatment of human ovarian carcinoma. *Cancer Chemother. & Pharm.* 18: 153-156, 1986.

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10. Reed, E., Ozols, R.F., Jarone, R., Yuspa, S.H., and Poirier, M.C.: Platinum-DNA adducts in nucleated peripheral blood cell DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy. *Proc. Natl. Acad. Sci. U.S.A.*, in press, 1987.

11. Ozols, R.F., Louie, K.G., Plowman, J., Behrens, B.C., Fine, R.L., Dykes, D., and Hamilton, T.C.: Enhanced alkylating agent cytotoxicity in human ovarian cancer in vitro and in tumor bearing nude mice by buthionine sulfoximine depletion of glutathione. *Biochem. Pharmacol.* 36: 147-153, 1987.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

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Molecular Biology of Hormone-Responsive Human Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Other:	Connie Agnor	Microbiologist	M	NCI
	Jim Voeller	Microbiologist	M	NCI
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	Francis Kern	Sr. Staff Fellow	M	NCI
	Susan Flamm	Medical Staff Fellow	M	NCI
	Maria Zajac-Kaye	Biotech. Train. Prog. Trainee	M	NCI
	Nils Brunner	Guest Worker	M	NCI
	David Levens	Sr. Investigator	LP	NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
9	9	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. We have conceived and constructed a method for cloning human cDNAs by virtue of phenotypic expression in human cells. This has been done in order to pursue the hormone dependent expression of genes from human breast cancer cells. An initial demonstration that this system works has been carried out in an experiment cloning a fibroblast growth factor gene by examining its expression in an indicator cell line which is absolutely dependent on a fibroblast growth factor activity for anchorage-independent growth. This FGF peptide is a novel peptide produced exclusively by breast cancer cells and is in the process of being sequenced and will be patented.

B. In conjunction with studies on fibroblast growth factor peptides in human breast cancer we have examined the expression of the four members of the FGF family, basic FGF, acidic FGF, int-2, and KS-FGF, in human breast cancer cell lines. We have found that the expression of fibroblast growth factor peptides correlates with estrogen unresponsiveness and may be a marker for malignant progression in breast cancer.

C. We have completed studies on the action of the ras oncogene in a model system which examines the tumorigenesis of human breast cancer cells in nude mice. We have concluded that activating mutations of the ras oncogene, which markedly diminish its GTPase activity, can enhance the tumorigenesis of this human breast cancer cell line, MCF-7. However, high levels of expression of the ras gene protein, a situation often seen in the clinical breast cancer, does not alter the tumorigenic behavior of these cells.

Jens Roosen	Guest Worker	M	NCI
David Levins	Laboratory of Pathology, DCBD	M	NCI

This model system does not support a role for the *ras* oncogene as being a determinant for the malignant behavior for breast cancer *in vivo*.

D. Studies with a second human hormone dependent malignancy, prostate cancer, have explored the effect of oncogenes in a hormone-dependent prostate cancer cell line, LNCaP. We have transfected both mutants of the *ras* oncogene and expression vectors for the transforming growth factor alpha (TGF-) into these cells to see the effect of these two genes on the hormone-independent growth and the tumorigenesis of this cell line. In both instances we have succeeded in getting the transfected gene expressed in the cells, however, we have not noticed substantial differences in the phenotype of the cells expressed in the transfected gene. However, *v-ras* transfected into LNCaP cells causes a 2-5 fold increase in the ability of the cells to grow in an anchorage-independent colony assay. No effect was seen on the tumorigenic potential of these cells in nude mice.

E. In collaboration with the Laboratory of Pathology we have identified a region in the first intron of the human *c-myc* oncogene which is frequently mutated in cases of human Burkitt's lymphoma. Mutations in this region alter the binding of a novel nuclear protein to a specific binding region in the DNA. This nuclear protein is a negative transcriptional factor and we believe that the mutations affecting the binding of this protein contribute to the deregulation of the *c-myc* gene in Burkitt's lymphoma and play a role in the genetic pathology and the contribution that *c-myc* makes to the etiology of Burkitt's lymphoma.

PUBLICATIONS

1. Wilding, G., Lippman, M.E., and Gelmann, E.P. LHRH agonists and human breast cancer cells. *Nature* 320-770, 1987.
2. Wilding, G., Lippman, M.E., and Gelmann, E.P. Effects of steroid hormones and peptide growth factors on protooncogene c-fos expression in human breast cancer cells. *Cancer Res.* 48: 802-805, 1988.
3. Freter, C., Lippman, M.E., Cheville, A., Zinn, S., and Gelmann, E.P. Alterations in phosphoinositide metabolism associated with 17-B estradiol and growth factor treatment of MCF-7 breast cancer cells. *Molec. and Cell. Endocrinol.* 2: 159-166, 1988.
4. Zajac-Kaye, M., Gelmann, E.P., and Levens, D. A point mutation in the *c-myc* locus of a Burkitt lymphoma abolishes binding of a nuclear protein. *Science* 240: 1776-1780, 1988.
5. Heckford, S.E., Gelmann, E.P., and Matis, L.A. Distinct mechanisms of *c-myc* and lymphokine gene expression in an antigen-specific T cell clone. *Oncogene* (in press), 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06714-02 M

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adoptive Cellular Immunotherapy of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael A. Bookman, M.D.	Senior Staff Fellow	MB, COP, DCT, NCI
Sandra Bridges	Cancer Expert	MB, COP, DCT, NCI
Eva Horak	Medical Technologist	MB, COP, DCT, NCI
Danny Dean	Biologist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Laboratory of Genetics, NCI
Bureau of Biologics, FDA
Biological Response Modifiers Program, NCI
Laboratory of Tumor Virus Biology, NCI

LAB/BRANCH

Medicine Branch

SECTION

Biological Therapeutics Section

INSTITUTE AND LOCATION

NIH, National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Adoptive Chemoimmunotherapy of Murine Leukemia. Cytolytic T lymphocyte (CTL) and helper T lymphocyte (HTL) clones have been established from tumor-bearing mice and evaluated in models of adoptive chemoimmunotherapy (ACIT) of syngeneic murine leukemia (RBL5). Antigen-specific clones have been utilized as tools for the investigation of factors important in mediating ACIT.

1. Characterization of the cellular immune response to retorvirus-induced leukemia. Tumor immunization promotes host HTL, CTL and suppressor cell immune responses. The majority of HTL are specific for viral envelope glycoprotein gp70, CTL recognize other tumor associated antigens (Ags) including gag. HTL produce interferon-γ (IFN-γ) and interleukin-2 (IL-2) after Ag stimulation, but are not directly cytolytic for tumor cells. In some cases, Ag-activated HTL transiently acquire the ability of lyse tumor cells *in vitro*.

2. Activation, dissemination, and survival of HTL after adoptive transfer. HTL can be activated *in situ* to elicit a delayed-type hypersensitivity (DTH) reaction after local transfer with tumor cells. The intensity of DTH is sufficient to prevent tumor outgrowth without exogenous factors. The mechanism by which non-cytolytic HTL mediate tumor rejection is uncertain, but may include recruitment and activation of host macrophages or NK cells. Participation of specific host cells is being studied by depletion of host subpopulations with monoclonal antibodies (MoAb). HTL can survive and expand in number after adoptive transfer if exogenous IL-2 and Ag are provided. Withdrawal of Il-2 is then associated with rapid reduction in cell numbers. HTL remain activated after transfer, and can be distinguished from normal host lymphocytes on the basis of size and

surface markers. Although HTL can enter lymphoid organs from the bloodstream, their capacity for normal recirculation is markedly reduced. HTL appear to be terminally differentiated effector cells that are not capable of reverting to small resting memory lymphocytes *in vivo*.

3. *Activity of HTL and CTL in ACIT.* Syngeneic mice with established peritoneal tumors received therapy with cyclophosphamide (CY) and transfer of clones. ACIT was not effective without CY. Transfer of HTL and CTL prolonged median survival and achieved long-term cures. Combinations of HTL+CTL appear synergistic. Control HTL specific for non-tumor Ags are not effective in ACIT and may actually interfere with anti-tumor responses. Exogenous IL-2 is not necessary for successful ACIT. Host peritoneal NK cells and macrophages are activated during ACIT with HTL. Participation of specific host cells is being studied by depletion of host subpopulations with MoAb.

4. *Augmentation of ACIT.* Pre-activation of host macrophages may enhance HTL or CTL anti-tumor responses. IFN-γ enhances median and long-term survival following ACIT with CTL. No direct effects of IFN-γ on tumor cells or CTL could be demonstrated. Administration of IFN-γ was associated with activation of host peritoneal NK cells and macrophages with increased tumor cytotoxicity. Muramyl tripeptide is a second activating agent undergoing evaluation *in vivo*.

5. *Mechanisms of tumor resistance to ACIT.* Not all mice are cured following ACIT with HTL or CTL. A number of ACIT-resistant tumors have been isolated for further characterization. A high frequency of resistant tumors express an altered form of gp70 Ag, and are not capable of activating certain HTL clones. However, all resistant tumors evaluated thus far have remained susceptible to CTL lysis.

6. *Pathways of T lymphocyte activation.* Activated lymphocytes are more readily expanded in number and are usually more potent as effector cells. Accessory surface molecules participate in conventional T cell activation (i.e. L3T4, Lyt2) or may provide alternative pathways of activation (i.e. Thy-1.2). MoAb directed at accessory molecules may either enhance or inhibit activation. We have demonstrated association of tyrosine kinase activity with the L3T4 accessory molecule on HTL clones.

Abridged References:

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Bookman MA, Swerdlow R, Matis LA (1987). Adoptive chemoimmunotherapy of murine leukemia with helper T lymphocyte clones. J. Immunol. 139:3166-70.

Veillette A, Horak ID, Horak EM, Bookman MA, Bolen JB (1988). Alterations of the lymphocyte-specific protein tyrosine kinase (p56lck) during T-cell activation. (submitted).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06715 01 M

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Tumor Cell Phosphoinositide Metabolism and Glucocorticoid Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Carl E. Freter, M.D., Ph.D.	Senior Staff Fellow	MB, COP, DCT, NCI
Sydelle Zinn	Medical Technologist	MB, COP, DCT, NCI

COOPERATING UNITS *(if any)*

Biological Response Modifiers Program (BRMP), Frederick, Maryland.
Laboratory of Pathology, NCI, NIH

LAB/BRANCH

Medicine Branch

SECTION

Biologic Therapeutic Section

INSTITUTE AND LOCATION

NCI, NIH, National Cancer Institute, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.25	0.75	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The following is an outline of clinical and laboratory activities during Fiscal Year 1988. Where applicable, I have indicated progress over this period of time with publications expected, in progress or submitted for each of these various projects.

Laboratory activities:

1. Phosphatidylinositol turnover in MCF-7 breast cancer cells in association with 17-β estradiol or growth factor treatment.

In this ongoing project, we have demonstrated that estradiol or TGF-α treatment of MCF-7 breast cancer cells is accompaned by elevated steady state levels of PI turnover, directly implicating TGF-α as an autocrine growth factor in these cells (TGF-β in contrast, is growth inhibitory and abrogates estradiol-induced increases in MCF-7 growth as well as PI turnover). In addition, we have characterized a phospholipase-C activity which initiates PI turnover and is elevated in estradiol treated and TGF-α treated cells. (The initial studies in this investigation have been published in Mol. Endocrinology 2:159-166, 1988).

2. Phosphoinositidemetabolism in LNCAP prostate cancer cells in response to dihydrotestosterone treatment.

These studies are similar to the above and demonstrate that hormonal treatment of prostate cancer cells is asssociated with elevated levels of PI turnover and that specific growth factors are able to mimic this change as well. (These studies have been carried out in collaboration with Dr. George Wilding and reported at the 1988 AACR meeting. A manuscript is in preparation).

3. Phosphoinositide metabolism in human melanoma cells treated with autcrine motolity factor.

Ongoing experiments with the Blise Kohn and Lance Liotta have indicated that autocrine motility factor operates through a phosphatidylinositol-dependent pathway. The results have been presented at the 1988 AACR meeting and a publication has been submitted. Other studies regarding the regulation of the phospholipase-C initiating phosphoinositide turnover in these cells are planned. In collaboration with the above investigators, studies have been undertaken using a novel inhibitor of g-protein activation which appears to have significant antitumor activity. Further laboratory studies using this compound are being planned, as well as a Phase I clinical protocol of this compound in the Medicine Branch.

4. Phosphoinositide metabolism in stimulated T-cell clones.

These experiments carried out in collaboration with Sue Hectford and Lou Matis as well as Ed Gellman, have inidcated that stimulation of phosphotidylinositol metabolism in resting T-cells by lectins, but not by IL-2, result in a 50-fold increase in phoshoinositide turnover, occuring about two hours after stimulation. Studies are underway to explore the relationship between glucocorticoids as well transforming growth factors particularly TGF-β with respect to their interaction with membrane signalling systems in lymphocyte activation. In addition, plans are underway to construct activated T-lymphocyte cDNA libraries to look for anonymous induced genes in the process of T-cell activation. The regulation of such genes will also be explored in the setting of HTLV retroviral infection.

5. Anti cell surface receptor antibodies (anti-EGF receptor):

Mechanisms of interrupting autocrine cell growth

In collaboration with Susan Bates and other memebers of Marc Lippman's laboratory, as well as Dr. Jeffrey Kudlow (University of Montreal), we have demonstrated the antibodies against the EGF receptor are able to abolish the majority of estrogen-induced growth in MCF-7 breast cancer cells and an initial publication containing this observation is in press. Other studies are planned to examine the effects of such antibodies on membrane signalling pathways as well as on *in vivo* growth in implanted in nude mice tumors.

6. Expression of EGF or EGF-like molecules in human breast cancer cell lines.

Using a full-length EGF-probe, several unique and previously undescribed message sequences have been detected in several breast cancer cell lines. Currently, efforts are being made to characterize such messages as well as attempts to immunoprecipitate EGF like material from such lines and culture media. Further studies may involve cloning and isolation of genomic forms of such messages to study the regulation of expression in these cell lines.

7. Activation of various ras oncogene alleles in MCF-7 breast cancer cells by infection with retroviral vectors.

In experiments in collaboration with Dr. Ed Gellman, we have used retroviral vectors containing various ras constructs from MCF-7 breast cancer cells to study their subsequent tumorogenicity, as well as steady state levels of phosphoinositide turnover. We have found that not all ras containing constructs induce alterations in PI turnover and efforts are currently underway to understand the mechanisms for ras induced induced phosphoinsoitide turnover in cell lines.

8. Membrane signalling in B-cell clones.

In collaboration with Drs. Longo and Kenny, early events in B-cell activation are being examined in monoclonal B-cell populations in transgenic mice. Membrane signalling events in B-cel interaction with antigen and antigen-presenting cells are being studied.

9. Construction and screening of human T-cell leukemia cDNA libraries to identify glucocorticoid-induced genes involved in cell killing.

This project is aimed at identifying and isolating glucocorticoid-induced genes involved in cell killing. cDNA libraries have been constructed from sensitive and resistant CEM human T-cell lymphocyte lines and subtraction hybridization techniques have been used to generate a number of clones that are either up or down regulated by glucocorticoids. These clones are undergoing continuing characterization and regulated clones will be further studied in detail including sequencing of their cDNAs.

9. Clinical protocol MB-218, a pilot protocol of intensive induction therapy and autologous bone marrow transplantation for high grade lymphomas.

This protocol has been written and implemented during this Fiscal Year and is aimed at improving curates in high grade lymphomas using intensive antileukemic induction therapy followed by autologous bone marrow transplantation with 4-hydroperoxycyclophosphamidebone marrow purging. Currently, eight patients have been treated on this protocol and all are too early early to evaluate. Several cell lines have been established from such patients and are being used to characterize changes in glucocortocoid sensitivity and responsiveness to other biological and antineoplastic agents.

PUBLICATIONS

1. Whang-Peng, J., Freter, C.E., Knutsen, T., Nanfro, J. and Gazdar, A.: Translocation (11, 22) in esthesioneuroblastoma. Cancer Genet. Cytognet., 29: 155-157, 1987.

2. Freter, C.E., Lippman, M.E., Cheville, A., Zinn, S., and Gelmann, E.P., Alterations in Phosphoinositide Metabolism Associated with 17-β Estradiol and Growth Factor Treatment of MCF-7 Breast Cancer Cells. Mol. Endocrinology, 2: 159-166, 1988.

3. Bates, S.E., Davidson, N.E., Valverius, E., Dixon, R.B., Freter. C.E., Tamm, J.P., Kudlow, J.E., Lippman, M.E. and Solomon, D.S.: Expression of Transforming Growth Factor α and Its MNRA in Human Breast Cancer: Regulation by Estrogen and Evidence for an Autocrine Role. Mol. Endocrinology, (in press).

4. Cohn, E.C., Stracke, M.L., Freter, C.E., Lippman, M.E., Schiffman, E. and Liotta, L.: Atuocrine Motility Factor Stimulates Phosphatidylinositol Turnover in Human Melanoma Cells. (submitted)

5. Wilding, G.W., Gelmann, E.P. and Freter, C.E.: Altered Phosphoinositide Metabolism in Response to Dihydrotestosterone Treatment of LnCAP Prostate Cancer Cells. (in preparation).

RECENT ABSTRACTS

1. Cohn, E., Stracke, M., Freter, C.E., Lippman, M.E., Schiffman, E. and Liotta, L.: Autocrine Motility Factor (AMF) Stimulates Phosphatidylinositol Turnover in Human Melanoma Cells. M. Assoc. Cancer Res. Proceedings. 259, 1988.

2. Aiba, K., Allegra, C.J., Park, J.G., Drake, J.C., Freter, C.E., Yeh, G.C. and Chabner, B.A.: An Investigation of the Determinants of 5-Fluorouracil (5-FU) Resistance in Human Colon Carcinoma Lines. American Assoc. of Cancer Res. Proceedings 1147, 1988.

3. Wilding, G., Gellman, E.P. and Freter, C.E.: Androgens Modulate Phosphotydalinositol Metabolism in Human Prostate Cancer Cells. American Assoc. of Cancer Res. Proceedings 926, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06716 01 M

PERIOD COVERED

November 1,1987 to September 30,1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Platinum drug resistance in human malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name. title. laboratory. and institute affiliation)*

Eddie Reed,M.D.	Sr. Investigator	MB/CPB,COP,DCT,NCI
Patricia Ormond,Ph.D.	Biotech Fellow	MB/CPB,COP,DCT,NCI
Janet Budd,B.S.	Chemist	MB/CPB,COP,DCT,NCI

COOPERATING UNITS *(if any)*

Lab of Cellular Carcinogenesis and Tumor Promotion, DCE,NCI
Laboratory of Molecular Pharmacology, DTP,DCT,NCI
U.S.C. Cancer Center, Los Angeles, California

LAB/BRANCH

Medicine Branch/Clinical Pharmacology Branch

SECTION

Office of the Chief, MB/CPB

INSTITUTE AND LOCATION

NIH,National Cancer Institute,Bethesda,Md. 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
2	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The Principal Investigator has shown that platinum drug resistance can be studied in cancer patients actively receiving chemotherapy. This project has been initiated to follow previously reported scientific leads into the mechanisms of platinum drug resistance in patients undergoing therapy. This project differs from the usual laboratory investigations in that the focus is drug resistance in the cancer patient. "Total man-years" above are estimated based on the following: the PI began independent work in November,1987 after lab renovations were completed; the laboratory technician started on January 4,1988; and the Biotech Fellow started on July 1,1988.

1. Cisplatin-DNA adduct can be measured in tissues of human cancer patients after therapeutic levels of drug exposure. Studies have been published by the PI to show that in ovarian and testicular cancer patients, adduct levels in leukocyte DNA correlates with disease response in both diseases, and that adduct levels in bone marrow closely approximate adduct levels in tumor tissues when specimens are obtained at autopsy. Studies are in progress to determine if disease response can be predicted in a prospective manner, based on the first two cycles of chemotherapy.

2. Assays are being developed to measure relative cisplatin-DNA adduct repair capacity in fresh human tissues. This method will utilize the recombinant DNA plasmid pRSVcat in a host cell reactivation assay, following defined levels of plasmid modification with cisplatin. Preliminary studies show that the assay differentiates between ovarian cancer cell lines with differing levels of cisplatin drug resistance. However, assay development is proceeding so as to increase the degree of resolution of the assay. Once assay development is completed, it will be applied to fresh human tissues from cisplatin protocol patients in a prospective study to assess the role of DNA repair in clinical cisplatin drug resistance.

3. A human DNA repair gene has been identified which has been transfected into UV-repair-deficient Chinese hamster ovary cells. This gene confers four to five fold resistance to cisplatin in this setting. The cDNA probe for this gene has been obtained, and we are beginning a cell line survey to assess the relationship, if any, between this gene and cisplatin sensitivity in human tumor cell lines. If a relationship between gene expression and cisplatin resistance becomes apparent in _in vitro_ studies, this tool will be used to assess clinical material as well.

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Reed, E., Litterst, C.L., Thill, C.C. Yuspa, S.H., Poirier, M.C.: Cisplatin-DNA adduct formation in renal, gonadal and tumor tissues of male and female rats. Cancer Res. 47:718-722, 1987.

Reed, E., Ozols, R.F., Tarone, R., Yuspa, S.H., Poirier, M.C. Platinum-DNA adducts in leukocyte DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy. Proc. Natl. Acad. Sci. USA, 84:5024-5028, 1987.

Poirier, M.C., Reed, E. Monitoring of human cancer patients for cisplatin-DNA adducts: A prototype for environmental and occupational exposures. Comments Toxicology, 1:307-316, 1987.

Litterst, C.L., Poirier, M.C., Reed, E. Factors influencing the formation and persistence of Pt-DNA adducts in tissues of rats treated with cisplatin. In Hacker,M.P., Lazo,J.S., and Tritton,T.R. (Eds) Organ Directed Toxcities of Anti-Cancer Drugs. Martinus Nijhoff Publishers, Boston, pp 159-171, 1988.

Reed, E., Poirier, M.C., Young, R.C., Ozols, R.F. High dose cisplatin in hypertonic saline: Toxicity versus therapeutic benefit. In Hacker,M.P., Lazo,J.S., and Tritton,T.R. (Eds.) Organ Directed Toxicities of Anti-Cancer Drugs. Martinus Nijhoff Publishers, Boston, pp 203-213, 1988.

Ozols, R.F., Hamilton, T.C., Reed, E., Poirier, M.C., Masuda, H., Lair, G., Young, R.C. High dose cisplatin and drug resistance: Clinical and laboratory correlations. In Nicolini, M. (Ed) Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy. Martinus Nijhoff Publishers, Boston, pp 197-206, 1988.

Poirier, M.C., Yuspa, S.H., Ozols, R.F., Egorin, M.J., Reed,E. Platinum-DNA adduct formation in vivo in tissues of cancer patients. Proceedings, International Conference on Reproduction and Human Cancer, Bethesda, MD, in press, 1988.

Reed, E., Sauerhoff, S., and Poirier, M.C. Quantitation of platinum-DNA binding in human tissues following therapeutic levels of drug exposure -- A novel use of graphite furnace spectrometry. Atomic Spectroscopy, 9:93-95, 1988.

Poirier, M.C., Egorin, M.J., Fichtinger-Schepman, A.M., Yuspa,S.H., and Reed, E. DNA adducts of cisplatin and carboplatin in tissues of human cancer patients. In Bartsch,H., Hemminki,K., and O'Neill,I.K. (Eds.) Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (IARC Scientific Publications No.89). pp 313-320, July, 1988.

Reed, E., Ozols, R.F., Tarone, R., Yuspa, S.H., Poirier, M.C. The measurement of cisplatin-DNA adduct levels in testicular cancer patients. Carcinogenesis, in press, 1988.

Reed, E., and Freireich, E.J. Cancer treatment and support programs for minorities: I. Treatment. Cancer Bull, 40:92-93, 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03024-19 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Clinical Trials and Other Clinical Investigations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	Stephen R. Veach, MD	Head, Hematology/Oncology Branch	NHBETH
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, PhD	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

COOPERATING UNITS (if any)
Radiation Oncology Branch; Biostatistics & Data Management Section; Surgical Oncology Division, Naval Hospital Bethesda; ematopathology Branch, Naval Hospital, Bethesda.

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Clinical Investigations

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5	2	3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although a dose-response curve clearly exists for alkylating agents in the initial chemotherapy of small cell lung cancer, the therapeutic benefit of higher than standard doses of the more recently introduced regimen of etoposide/cisplatin (VP16/PLAT) is uncertain. We randomized at least partially ambulatory patients with extensive stage SCLC and and without major organ dysfunction to received either VP16 80 mg/m squared Days 1-5 q 3 wks or VP16 80 mg/m squared Days 1-3 + PLAT 80 mg/m squared Day 6 1 q 3 wks for the first 6 wks of therapy. Nonambulatory patients and those with organ dysfunction were assigned standard dose treatment. All patients received the standard dose regimen during wks 7-12. From wks 13-24, patients in complete response (CR) continued standard dose VP16/PLAT, while all other patients received a new 3-drug regimen that led to further improvement in response in only 4 cases. CR's were given prophylactic cranial irradiation. Seventy-eight patients have been entered (61 of whom were randomized). With a median follow-up of 33 mos, preliminary results are:

	N	CR	CR+PR	Med Surv	Nadir WBC	Nadir Plt
High	28	27%	81%	13 mos	1,400	50,000
Standard	33	26%	84%	11 mos	2,600	179,000
Nonrad	17	6%	71%	6 mos	1,800	90,000

CR rates (p=1.00) and survival (p=0.54) were similar in patients randomized to high and standard dose therapy. There were 2 treatment-related deaths in the high and non in the standard dose arm. We conclude 1) standard dose VP16/PLAT is at least as active as any regimen we have ever utilized for for extensive stage SCLC and produces only modes myelotoxicity, and 2) there is no evidence of superior efficacy when planned drug doses are increased by 67% during the first 6 wks.

PROJECT DESCRIPTION

Clinical Trials and Other Clinical Investigations

Professional Staff:

PI:	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	R. Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	Stephen R. Veach, MD	Head, Hematology/Oncology Branch	NHBETH
	John D. Minna, MD	Chief, NCI-NMOB	NCI-NMOB
	Herbert K. Oie, PhD	Microbiologist	NCI-NMOB
	Edward K. Russell	Chemist	NCI-NMOB

Collaborating Branches:

Eli Glatstein and Jane Grayson, Radiation Oncology Branch; Seth M. Steinberg, Biostatistics & Data Management Section; Bimal Ghosh, Surgical Oncology Division, Naval Hospital Bethesda; James Cotelingham, Hematopathology Branch, Naval Hospital, Bethesda.

Objectives, Rationale, and Background:

This trial has several objectives. We wished to determine in a prospective randomized fashion whether high doses of etoposide (VP16) and cisplatin (PLAT) given during a six-week induction period would produce higher complete response rates and better survival than standard doses of the same drugs in patients with extensive stage small cell lung cancer (SCLC). We also wished to assess the feasibility and value of individualized chemotherapy selection based upon in vitro drug testing of tumor cell lines derived from pre-treatment patient tumor specimens. Objectives of this portion of the study were to determine the frequency with which tumor-containing specimens can be obtained from unselected patients with extensive stage SCLC, the frequency of successful cell culture and drug sensitivity testing, the degree of heterogeneity of drug sensitivity among different cell lines the correlation between in vitro drug sensitivity and clinical response, and the clinical utility of individualized drug selection based upon in vitro data.

The introduction of combination chemotherapy into the management of SCLC has led to four- to five-fold improvement in median survival and five-year disease-free survival in a small fraction of patients. Although median survival is improved to approximately the same degree compared to untreated patients in limited stage and extensive stage disease, survival of two years or more only rarely occurs in patients with extensive disease, defined as tumor extending beyond the hemithorax of origin and the regional lymph nodes. Furthermore, chest irradiation has never been suggested to yield any survival benefit in extensive stage patients. Therefore, virtually all patients with extensive SCLC are suitable subjects for investigational chemotherapy studies.

Methods Employed:

Moderately aggressive chemotherapy which produces leukopenia in the range of 1,000/mcl has been shown to be superior to less intensive treatment that is virtually never associated with leukopenic fever in both randomized and non-randomized studies. However, even more intensive initial (or induction) therapy which is so myelosuppressive that hospitalization of all patients is required has not been demonstrated to provide additional benefit, although randomized studies have not addressed this issue. In most of these studies, the drugs given in very high doses have been to provide additional benefit, although randomized studies have not addressed this issue. In most of these studies, the drugs given in very high doses have been restricted to cyclophosphamide, doxorubicin, and VP16. VP16/PLAT has been shown to be a highly synergistic combination regimen in treatment of murine leukemia and in early studies appears to be as active as most three- or four-drug combinations in patients with SCLC. VP16/PLAT is also more active than VP16 alone as a salvage regimen in this tumor. PLAT in higher than conventional doses appears to have increased activity in testicular and perhaps ovarian cancer. Although higher than standard doses of VP16/PLAT have been employed in small studies in SCLC, the issue of dose-response with this combination has not been addressed in a prospective randomized trial. We therefore initiated such a study. The first four patients randomized to the high dose regimen received VP16 120mg/m^2 x 5 and PLAT 40mg/m^2 x 5. Two died of infection before Day 21 without recovery from myelosuppression, and the doses of drugs on the high dose arm were subsequently reduced to VP16 80mg/m^2 x 5 and PLAT 27mg/m^2 x 5. Throughout the trial, doses on the standard arm have been VP16 80mg/m^2 x 3 and PLAT 80mg/^{2} x 1. Since a significant minority of extensive stage SCLC patients are not candidates for a very myelosuppressive regimens, such patients (deemed "poor risk") are not randomized but rather assigned to standard dose therapy.

For the past 10 years, the human tumor stem cell assay of Hamburger and Salmon has been most commonly employed for in vitro drug testing of human cancer. In applying this test to fresh tumor specimens from our SCLC patients, however, we found that sufficient tumor colonies for adequate in vitro testing of even a single drug were present only 23% of the time. Clearly, different approaches were needed to apply in vitro drug testing to a large fraction of patients. Since our laboratory has considerable experience in establishing permanent cell lines of SCLC, we decided to utilize cell lines rather than fresh tumors for drug testing. Compared to fresh tumors, cell lines provide tumor cells that are free of contaminating stromal cells and can be subjected to repeated testing. The time from specimen procurement to assay results, however, is delayed.

A modification of the Weisenthal dye exclusion assay was employed for drug testing because the assay is technically simple, does not require a single cell suspension, can be completed in four days, and can be applied to many tumors and most cell lines. Reading the assay, however is labor intensive and subjective and can be confounded by cell clumping.

Major Findings:

Sevety-eight patients have been entered. Median follow-up from time of patient entry is approximately 33 months. Seventeen of the 78 patients were assigned standard dose therapy because of poor performance status, brain, lung or cardiac dysfunction, or refusal to be randomized. The remaining 61 were randomized to receive high or standard dose VP16/PLAT for the first 6 weeks of therapy.

On the high dose arm, 21 (81%) of 26 have responded to therapy, including 7 (27%) complete responders, and actuarial median survival is 13 months. On the standard dose arm, 26 (84%) of 31 patients responded, including 8 (26%) complete responders, and actuarial median survival is 11 months. There is no significant difference between the two groups in complete response rate ($p = 1.00$) or overall survival by the logrank test ($p = 0.54$). As expected, the response rate (6% complete, 65% complete plus partial) and survival (actuarial median 6 months) are inferior in patients judged not suitable for randomization. Among all 78 patients, performance status and number of distant organ systems involved with metastatic disease (0-2 vs. 3-7) are highly significant predictors of survival ($p < 0.001$ and $p = 0.009$, respectively).

Hematologic toxicity is significantly worse on the high dose induction program (median nadir WBC count 1,400/mcl and platelet count 5,000/mcl) compared with the standard dose induction (median nadirs 2,600 and 179,000, respectively). Among the poor risk nonrandomized patients, median nadir WBC count has been 1,800/mcl and median nadir platelet count, 90,000/mcl. There have been five treatment-related deaths, all due to myelosuppression and infection, two on the high dose arm prior to lowering of the drug doses and three in poor risk patients assigned standard dose therapy. Although only 33 patients have been treated, the standard dose regimen yields results at least as good as our historical experience in good risk extensive stage SCLC with considerably less hematologic toxicity, suggesting it may have a superior therapeutic index.

A total of 124 pre-treatment staging specimens have been submitted for cell culture from these 63 patients (1.8/patient). Sixty-six specimens (53%) contained tumor cells. Twenty-four cell lines, defined as sufficient in vitro amplification of tumor cell number to allow testing of multiple drugs in duplicate at three concentrations have been obtained. The largest numbers of positive specimens and cell lines were derived from bone marrow, peripheral lymph nodes, and pleural effusions. Procurement of only five specimens required administration of general anesthesia, but three of these five procedures were performed for diagnostic purposes. Among the 69 patients with a minimum six-month follow-up, at least one staging specimen reached the cell biology laboratory in 68 (99%), and a tumor-containing specimen was procured from 52 (75%) of these previously untreated patients. A cell line was obtained from 24 (35%), or 46% of patients from whom a tumor-containing specimen was available. In addition, tumor-containing specimens have been obtained from 16 of these patients after tumor progression on chemotherapy, and a cell line has been successfully grown from eight.

Actuarial median survival of patients from whom a tumor cell line was successfully grown, patients from whom a tumor-containing specimen was obtained but did not grow in vitro, and patients from whom no tumor-containing specimen could be procured was 7, 11, and 17 months respectively. Patients with no tumor specimen had superior survival by the logrank test ($p < 0.02$). The survival of patients whose tumor specimens were or were not successfully cultured was not significantly different ($p = 0.72$). Thus, whether a patient had sufficient tumor dissemination that a biopsy specimen could be relatively easily obtained was of greater prognostic import than whether a cell line could be established from a positive biopsy specimen.

In vitro drug testing has been completed on tumor cell lines derived from 22 previously untreated SCLC patients. In vitro drug sensitivity of these cell lines correlated extremely well with response to therapy to VP16 PLAT. In 13/14 (93%) lines from patients with complete or partial response at 12-week restaging, two or more drugs were "active." Sensitivity patterns were strikingly different in the five lines from patients who never responded to VP16/PLAT or had progressed by Week 12. In none of these lines were two or more "active" drugs identified, and only 2/33 (6%) of individual drug assays indicated "activity." P values for these differences were 0.001 and <0.0001, respectively. For each of the seven drugs considered individually, lines from responding patients always exhibited a lower mean cell survival at the reference concentration than lines from non-responding patients. Evaluation of these differences with the 2-sample rank yielded p values of less than 0.05 from VP16, doxorubicin and mechlorethamine, and less than 0.10 for vincristine.

Complete response rates to the first chemotherapy regimen given after VP16/PLAT were compared in patients receiving an "in vitro best regimen" based on in vitro drug testing, or in those receiving vincristine/doxorubicin/cyclophosphamide (VAC) when in vitro drug testing results were not available for whatever reason. Thirty-one patients were treated with VAC after failure to achieve complete response by Week 13, and five after relapse from complete response induced by VP16/PLAT. In these 36 patients, there were two complete responses (6%). Among the 15 patients who received their "in vitro best regimen," 12 had failed to achieve complete response at Week 13, and three had relapsed. Four patients (27%) attained complete response to their chemotherapy program based on in vitro drug testing (p = 0.11, Fisher´s exact test).

Significance to Biomedical Research and the Program of the Institute:

Thus far, there is no indication from this study that a high dose regimen of VP16/PLAT (67% higher doses of each drug, 44% higher doses/unit time actually administered) is in any way superior to standard doses of this two-drug regimen. On the other hand, the standard dose program is well tolerated and may be as effective as any other SCLC regimen, based on this data and that of others. Given the low complete response rate to any of the drug programs given to partial or non-responders at Week 13, it is likely that most or all of the survival benefit our patients received from therapy produced solely by VP16/PLAT.

The interim results of this trial serve to emphasize several problems that arise in implementing a program of individualized chemotherapy selection with our current technology and study design. First, procurement of tumor specimens, establishment of cell lines, and drug testing are extremely labor intensive and time consuming. More efficient assay techniques and better understanding of the relationship between in vitro and in vivo pharmacokinetics would be valuable. Second, drug testing has been possible in only one-third of patients, and improved methods of cell culture are still needed. We believe these interim results justify the more frequent employment of major surgical procedures to procure larger, more rapidly grown tumor specimens in good risk consenting patients, and have already begun such a program in limited stage patients, who would be expected to more frequently be able to tolerate elective general anesthesia. And third, with the time required to establish and perform drug testing on cell lines, treatment based on in vitro testing can often be given only 10 to 12 weeks after a tumor specimen is obtained and may not be relevant to the in vivo drug sensitivity pattern present in residual tumor cells present at that time. Procurement of larger tumor specimens could help to alleviate this problem and allow more rapid drug testing and quicker administration of "individualized" chemotherapy.

Proposed Course:

In a statistical analysis done two months ago, when 57 patients with follow-up had been randomized, 95% confidence limits for differences in 12-month survival ranged from favoring the high dose arm by as much as 38% to favoring the standard dose arm by as much as 17%. If the "true" complete response rates were 25% and 50% for standard and high dose therapy, respectively, entering 20 more patients per arm would have only 11% probability of detecting this stated difference with a one-sided 5% test. If the "true" probabilities were 20% and 30%, respectively, 20 more patients per arm would result in only a 0.7% probability of detecting a significant result. An evaluation of the same issue using a survival rather than a response end point is now ongoing. Continuing randomization to high or standard dose therapy will be reassessed when it is complete.

Despite these problems and the preliminary nature of our results, we believe several conclusions are justified. First, results of drug sensitivity testing of tumor cell lines are highly correlated with response to initial chemotherapy. Second, preliminary results utilizing in vitro drug testing for individualized selection of chemotherapy regimens suggest modest potential for therapeutic benefit. Third, the close correspondence between in vitro and in vivo response to drugs provides justification for the use of human cancer cell lines in screening for new chemotherapeutic agents. And finally, the availability of multiple SCLC tumor cell lines from patients whose clinical course is well characterized, including some paired lines from patients before and after in vivo chemotherapy, may prove useful in helping to elucidate the basis for drug resistance and other biologic properties of this tumor.

Publications:

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2. Ihde DC, Russell EK, Oie HK, Linnoila RI, Steinberg SM, Ghosh BC, Schumacher HR, Minna JD, Gazdar AF. Prospective clinical trial of individualized chemotherapy based on in vitro drug sensitivity testing in extensive stage small cell lung cancer. In: Salmon SE, ed. Adjuvant Therapy of Cancer V. Orlando: Grune & Stratton, 1987;201-07.

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Objectives:

1. To obtain from a prospective staging study and longitudinal follow-up information bearing on the natural history and prognosis of cutaneous T cell lymphomas (Mycosis fungoides (MF) with or without the Sezary syndrome).

2. To obtain material in approved staging procedures from patients to explore the molecular and cellular basis of post-thymic malignancies.

3. To continue the follow-up and ascertain the effects of treatment on patients previously entered on a protocol to study the effect of combined modality (electron beam and combination chemotherapy) on MF.

4. To continue the follow-up and ascertain the effects of treatment on patients previously entered on a randomized trial comparing aggressive combined modality therapy (electron beam radiation therapy and combination chemotherapy) vs. sequential topical, conservative therapies.

5. To accession patients onto a study which randomizes newly diagnosed, early stage patients between electron beam radiation therapy with or without chemotherapy, followed by adjuvant topical nitrogen mustard.

6. To accession patients onto a study of a novel, T cell targeted alternating phase II regimen - deoxycoformycin and interferon - after they have failed prior therapies or *de novo* if of advanced stage.

7. To explore novel salvage therapies in patients who have failed the approach outlined in Item 6.

Mycosis fungoides and the Sezary syndrome share common cutaneous histopathologic features, and this spectrum of malignant disease is referred to here as cutaneous T-cell lymphoma (CTCL). A method (LN classification) for describing the histopathologic features of lymph nodes in CTCL is presented. In this system, lymph node biopsy specimens are scored according to the number of atypical lymphoid cells in T-cell-dependent paracortical zones and the preservation or distortion of the lymph node architecture. Lymph node architecture is preserved in lymph nodes scored LN1 to LN3, and these nodes may have coexistent dermatopathic change. LN1 nodes have single infrequent atypical lymphocytes in paracortical T-cell regions. LN2 nodes have small clusters of paracortical atypical cells. LN3 nodes have large clusters of atypical cells. LN4 nodes are partially or totally effaced by atypical cells. This system was used to classify 96 lymph node biopsy specimens obtained within six months of the initial diagnosis of CTCL; no LN1 nodes, 37 LN2, 44LN3, and 15 LN4 nodes were found. The LN class was significantly correlated with the extent of skin, blood, and visceral involvement, as well as with survival. Patients with LN2 lymph nodes have an estimated five-year survival of 70 per cent, while patients with LN3 and LN4 nodes have estimated five-year survivals of 30 and 15 per cent, respectively. The survival differences between the LN subgroups were all significant ($P<0.05$). The LN classification system was clearly shown to be reproducible among experienced pathologists. The LN system for the histopathologic classification of lymph nodes in CTCL is of prognostic value and should be used to assess lymph node biopsies in patients with CTCL.

Peripheral blood lymphocyte morphology was evaluated prospectively by light microscopy of blood smears and E rosette preparations in 160 patients with cutaneous T cell lymphoma (CTCL). Blood involvement was related to the type of cutaneous T-stage, being present in 90% of patients with erythroderma (T4), 27% of those with cutaneous tumors (T3), 9% of those with generalized (T2), and 0% of those with limited skin plaques (T1). Untreated patients with blood involvement (38 of 105) had a higher frequency of CTCL in lymph nodes and viscera and survival inferior to that of patients with normal or nondiagnostic lymphocyte morphology ($P<.001$). Multivariate analysis showed skin stage and age to be the most important pretreatment risk factors for survival. Although blood involvement was not an independent risk factor for the entire group, it appeared to have some adverse influence in the T2/T3 subsets ($P=.051$). Both lymphocytosis and size distribution of the circulating CTCL cells at initial diagnosis influenced survival. Patients with "mixed cell" cytology (>20% large [>11 m] CTCL cells), had a worse survival than those with predominantly small circulating CTCL cells ($P=.009$). The former were more likely to have aggressive features, including lymph node effacement by tumor ($P<.001$) and visceral disease ($P=.074$), than were "small cell" patients. Our data indicate that detailed review of the blood lymphocyte morphology in patients with diagnosed or suspected CTCL is helpful in predicting extent of disease and prognosis.

The clinical course of cutaneous T cell lymphoma (mycosis fungoides and Sezary syndrome) is generally indolent, but in occasional patients becomes fulminant. We found that biopsies from patients with accelerating disease can reveal cytologic transformation from previously observed small, convoluted lymphocytes to large cells that are similar to cells seen in large-cell lymphoma. The cerebriform nuclei characteristic of malignant T cells can only rarely be identified. Of 150 cutaneous T cell lymphoma patients we treated from 1976 to 1984, cytologic transformation was identified in 12 after review of peripheral blood smears and biopsies from skin, lymph nodes, and visceral sites. Patients who developed cytologic transformation were initially characterized by advanced stage (11 of 12), with lymph node effacement (seven of 11) and erythroderma (five of 12). The tumor cell DNA content after transformation was aneuploid (four of four), and the ability to form rosettes with sheep erythrocytes was retained in transformed cells (three of three). The median time from diagnosis of cutaneous T cell lymphoma to cytologic transformation was 21.5 months (range, 0 to 19+). We conclude that cytologic transformation in cutaneous T cell lymphoma represents a distinct clinicopathologic entity, characterized by an aggressive clinical course.

Study Objective. To determine the optimal staging evaluation at the time of initial diagnosis of patients with mycosis fungoides or the Sezary syndrome.

Design. Retrospective review of a uniformly staged inception cohort.

Setting. Single-institution tertiary care center.

Patients. Initial evlauation of 152 consecutive patients with mycosis fungoides with or without the Sezary syndrome within six months of initial definitive diagnosis.

Intervention. A detailed staging evaluation including physical examination, routine laboratory studies, chest X-ray, lymphangiogram evaluation of the peripheral smear, lymph node biopsy, bone marrow aspirate/biopsy, and liver biopsy in selected patients.

Measurements and main results. Univariate adverse prognostic features at initial diagnosis in patients with mycosis fungoides included ($p<0.01$) one or more cutaneous tumors or generalized erythroderma; adenopathy; blood smear involvement with Sezary cells; lymph node effacement; eosinophilia; and visceral involvement. Important and clearly independent prognostic factors in a multivariate analysis are the presence of visceral disease and the type of skin involvement.

Conclusion. A staging system based on histopathologic evaluation of skin, nodes, blood, and visceral sites provides more comprehensive prognostic information than clinical evaluation of skin disease and adenopathy. Based on the prognostic factors listed above, patients may be divided at initial presentation into three prognostic groups: good-risk patients have plaque-only skin disease without node, blood,k or visceral involvement (median survival in excess of 12 years); intermediate-risk patients have cutaneous tumors, erythroderma, or plaque disease with node or blood involvement but without visceral disease or node effacement (median survival of 5 years); and a poor-risk group with visceral involvement or node effacement (median survival of 2.5 years).

Thirty-nine patients with cutaneous T cell lymphoma (CTCL; including mycosis fungoides or the Sezary syndrome) with no previous treatment other than topical therapy or oral corticosteroids, received total skin electron beam irradiation (TSEB) and either sequential or simultaneous systemic chemotherapy. Median follow-up, measured from the time of initiation of therapy to the time of analysis, is in excess of 6 years and extends to 100+ months. Thirteen patients with stage I disease (limited to skin with no adenopathy) received 3,000 rad total skin electron beam irradiation followed by three 2-week courses of daily intravenous (IV) mechlorethamine. Twenty-six patients with advanced disease (stage II-IV) received 2,400 rad of TSEB and simultaneous chemotherapy with two alternating three-drug regimens: vinblastine, doxorubicin, and bleomycin (VAB) alternating with cyclophosphamide, methotrexate, and prednisone (CMP) administered over 54 weeks. The overall response rate was 92% with 16 of 39 patients (41%) achieving a histologically documented complete response (CR). Stage I patients had a significantly increased CR rate (77%) compared with stage II-IV ($P<.01$). The overall 6-year survival was 92% for stage I patients and 26% for stage II-IV patients (23%) ($P<.001$). Among ten completely responding stage I patients, six remain alive and disease-free survival is 26 months for completely responding stage II-IV patients ($P=.04$), but none are continuous disease-free survivors after protocol treatment. We conclude that combined modality treatment can be safely administered and produces prolonged disease-free survival in some stage I patients, but not in more advanced stage patients.

Publications:

1. Sausville EA, Worsham GF, Matthews MJ, Makuch RW, Fischmann AB, Schechter GP, Gazdar AF, Bunn PA. Histologic assessment of lymph nodes in mycosis fungoides/Sezary syndrome (cutaneous T-cell lymphoma): Clinical correlations and prognostic import of a new classification system. Hum Pathol 1985;16: 1098-1109.

2. Schechter GP, Sausville EA, Fischmann AB, Soehnien F, Eddy J, Matthews MJ, Gazdar A, Guccion J, Munson D, Makuch R, Bunn PA. Evaluation of circulating malignant cells provides prognostic information in cutaneous T cell lymphoma. Blood 1987;69:841-849.

3. Dmitrovsky E, Matthews MJ, Bunn PA, Schechter GP, Makuch RW, Winkler CF, Eddy J, Sausville EA, Ihde DC. Cytologic transformation in cutaneous T cell lymphoma: A clinicopathologic entity associated with poor prognosis. J Clin Oncol 1987;5:208-215.

4. Winkler CF, Sausville EA, Ihde DC, Fischmann AB, Schechter GP, Kumar PP, Nibhanupdi JR, Minna JD, Makuch RW, Eddy JL, Bunn PA. Combined modalilty treatment of cutaneous T cell luymphoma: Results of a 6-year follow-up. J Clin Oncol 1986;4:1094-1100.

5. Sausville EA, Eddy J, Makuch RW, Fischmann AB, Schechter GP, Matthews MJ, Glatstein E, Ihde DC, Kaye F, Veach SR, Phelps R, O'Connor, T, Trepel JB, Cotelingam JD, Gazdar AF, Minna JD, Bunn PA. Histopathologic staging at initial diagnosis of mycosis fungoides and the Sezary syndrome: Definition of three distinctive prognostic groups. Ann Int Med, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06575-13 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oncogenes, Chromosomal Deletions & Growth Factors in the Pathogenesis of Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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12	10	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nuclear acting cellular proto-oncogenes have been studied in lung cancer lines and primary tumor specimens of all histologic types. These have led to the discovery of a series of DNA and RNA abnormalities showing that these play a key role in the pathogenesis of lung cancer. Recent work has led to the discovery of: new types of genetic changes including alterations in transcription and loss of attenuator function for myc family members; characterization of the protein products and biologic activity of the genes through transfection studies; and identification of the role of the c-jun gene in lung cancer.

A considerable effort is going into the cloning and characterization of chromosomal deletion and translocation abnormalities in lung cancer cells using molecular genetic and cytogenetic techniques. There is a particular focus on the 3p deletion region, and the rb gene product which was found to be abnormal in the large majority of small cell lung cancers.

A series of peptides produced by lung cancer cells including insulin-like and transferrin-like growth factors, opioid peptides, and their receptors, as well as GRP and other peptide hormone gene associated peptides have been identified as being autocrine growth factors involved in the pathogenesis of lung cancer. Genetic changes involved in the pathogenesis of human lung cancer including oncogene activation, chromosomal deletions, and autocrine growth factor production.

To identify and characterize the genetic changes (somatic and constitutional) and mechanisms leading to the pathogenesis of lung cancer and to use this information to develop new methods to prevent, diagnose, and treat lung cancer.

PROJECT DESCRIPTION

Oncogenes, Chromosomal Deletions & Growth Factors In the Pathogenesis of Lung Cancer

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Project #1: DNA Status, Expression, and Protein Products of Nuclear Acting Proto-Oncogenes in the Lung Cancer Cells

The products of the L-myc gene, first discovered in lung cancer, have been characterized as short lived, nuclear phosphoproteins, derived from several alternatively processed mRNAs, that in some cases initiate off novel non-AUG codons. (DeGreve, Kaye, Nau, Birrer, Battey and Minna)

The various products of the L-myc gene have been shown to have biologic activity in transfection assays of transforming normal rat embryo cells and non-malignant rat lines to malignancy. (Birrer and Minna)

The molecular mechanisms of expression of c-myc, N-myc, and L-myc have been characterized in small cell lung cancer and have been shown to be related to differences in initiation of transcription, and loss of attenuator functions. This was the first characterization of these properties for N-myc and L-myc, and the first study of this in a defined tumor type. These studies show new mechanisms of genetic changes for activation of myc family genes in lung cancer. (Krystal, Nau, Minna and Battey)

The newly described oncogene c-jun (related to transcription factor AP-1) was found to be expressed at very high levels in lung cancer cells of all histologic types as well as in normal lung. Because of the relationship of c-jun with tumor promotor action, this finding predicts a unique scenario for tumor promotion in the lung. A series of transfection studies are being done to characterize the biologic activity of this gene. (Schutte, Birrer and Minna)

A series of new anti-peptide antisera to characterize the L-myc proteins have been prepared and are being used to further characterize these proteins. A series of in vitro mutants of the L-myc gene have been prepared to test for biologic activity of different forms of the L-myc proteins.

Project #2: Identification and Characterization of Chromosomal Deletion or Recessive Oncogenes

Retinoblastoma (rb) gene abnormalities occur in small cell lung cancer and pulmonary carcinoids with 50% having 13q14 cytogenetic abnormalities and 20% exhibiting DNA abnormalities. At the mRNA level, 80% had rb abnormalities (60% showing absent rb mRNA expression, and another 20% having greatly reduced levels). These studies show that the recently described rb gene plays a significant role in a common adult malignancy. We plan to characterize the protein product and biologic activity of the rb gene in lung cancer cells. (Harbour, Kaye, Gazdar, and Minna)

Restriction fragment length polymorphism (RFLP) analysis has shown loss of heterozygosity for markers in the 3p and other chromosome regions in lung cancer. In addition, new cytogenetic studies show a variety of previously unrecognized chromosomal deletions and translocations in lung cancer. These studies are leading the way to discovery of new tumor suppressor (chromosome deletion) genes in lung cancer. (Johnson, Kaye, Gazdar and Minna in collaboration with S. Naylor, Univ. Texas, B. Zbar, FCRC, and J. Whang-Peng, MB, CC, NIH)

A series of strategies are being used to isolate chromosome deletion genes including: subtractive cDNA cloning; subtractive DNA cloning (using the PERT technique); and chromosomal walking from known RFLP probes. For example, we have cloned and partially sequenced a cDNA from the 3p21 RFLP region. (Minna, Nau, Takahashi, Harbour amd Kaye)

Project #3: Identification of Autocrine and Other Growth Factors used by Lung Cancer Cells

An insulin like growth factor (most similar to IGF-1) functions as an autocrine growth factor for small cell and non-small cell lung cancer providing a new potential target for anti-tumor therapy. (Cuttitta, Mulshine and Minna in collaboration with R. Natale, Univ. Michigan)

GRP gene associated peptides (GGAPs) predicted from molecular biologic studies, and demonstrated to exist by anti-peptide antibodies, have also been found to have receptors on lung cancer cells and biologic activity in stimulating the growth of lung cancer cells in vitro. These observations provide another target for anti-autocrine growth factor therapy. (Cuttitta and Minna in collaboration with R. Natale, Univ. Michigan, and John Walsh, UCLA)

The presence of specific opioid receptors (mu, delta, kappa, sigma) has been found in lung cancer cells and differences between small cell and non-small cell lung cancer described. Of great interest, exogenously added opioids (such as morphine) will inhibit the growth of lung cancer cells in vitro with appropriate receptor specificity. This provides a new treatment strategy based on opioids in lung cancer patients, not only to relieve pain, but also to shrink tumors. (Maneckjee and Minna)

To identify and characterize new autocrine growth factors we are: testing downstream peptides from known peptide hormone genes produced by lung cancer cells such as those related to IGF-1; fractionating and characterizing peptides from serum free conditioned media of lung cancer cells adapted to grow in serum and exogenously added hormone free medium; using cDNA techniques to clone genes in expression vectors. (Cuttitta, Kasprzyk, Mulshine and Minna)

Publications:

1. Kaye F, Battey J, Nau M, Brooks B, Seifter E, DeGreve JS, Birrer M, Sausville EA, Minna JD. Structure and expression of the human L-myc gene reveal a complex pattern of alternative mRNA processing. Mol Cell Biol 1988;8:186-195.

2. Johnson BE, Sakaguchi AY, Gazdar AF, Minna JD, Burch D, Marshall A, Naylor SL. Restriction fragment length polymorphism studies show consistent loss of chromosome 3p alleles in small cell lung cancer patients′ tumors. J Clin Invest, in press.

3. Birrer MJ, Segal S, DeGreve JS, Kaye F, Sausville EA, Minna JD. L-myc cooperates with ras to transform primary rat embryo fibroblasts. Mol Cell Biol 1988;8:2668-2673.

4. Krystal G, Birrer M, Way J, Nau M, Sausville EA, Thompson C, Minna JD, Battey J. A block to transcription elongation is an important mechanism for regulating c-myc and L-myc mRNA expression in small cell lung cancer. Mol Cell Biol, in press.

5. Nakanishsi Y, Mulshine J, Kasprzyk PG, Natale RB, Maneckjee R, Avis I, Treston AM, Gazdar AF, Minna JD, Cuttitta F. Small cell lung cancer cells autostimulate their growth via an insulin-like growth factor-I activity: Evaluation of four cell lines. J Clin Invest, in press.

6. Harbour JW, Lai S-L, Whang-Peng J, Gazdar AF, Minna JD, Kaye F. Abnormalities in structure and expression of the human retinoblastoma gene in small cell lung cancer. Science, in press.

7. Minna JD, Cuttitta F, Battey JF, Mulshine JL, Linnoila I, Gazdar AF, Trepel J, Sausville EA. Gastrin-releasing peptide and other autocrine growth factors in lung cancer: Pathogenetic and treatment implications. In: DeVita VT, Hellman S, Rosenberg SA, eds. Imp Adv Oncol. Philadelphia: JB Lippincott 1988;55-64.

8. Minna JD, Battey J, Birrer M, Cuttitta F, DeGreve JS, Gazdar AF, Ihde D, Johnson BE, Kaye F, Krystal G, Lebacq AM, Linnoila I, Mulshine JL, Nau M, Sausville EA, Schutte J, Trepel J. Genetic changes involved in the pathogenesis of human lung cancer including oncogene activation, chromosomal deletions and autocrine growth factor production. In: Fortner JG, JE Rhoads, eds. Accomplishments in Cancer Research 1987, General Motors Cancer Research Foundation. Philadelphia: JB Lippincott 1988;155-182.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06578-05 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Biology of the Mammalian GRP Gene Family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James F. Battey, MD, PhD	Senior Investigator	NCI-NMOB
Others:	Anne-Marie Lebacq-Verheyden, MD, PhD	Guest Researcher	NCI-NMOB
	Geoffrey Krystal, MD, PhD	Medical Staff Fellow	NCI-NMOB
	James Way	Microbiologist	NCI-NMOB

COOPERATING UNITS *(if any)*
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LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology and Immunology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure, function, and expression of mammalian prepro gastrin releasing peptide (GRP) gene was studied. Experiments were performed to a) characterize the structure and regulation of the rat prepro GRP gene, b) overexpress the human GRP prohormone protein to analyze post-translational processing and peptide hormone function, c) obtain cDNA clones for other bombesin family prohormone genes.

A. Rat prepro GRP gene. The structure and entire nucleotide sequence of the rat prepro GRP gene was determined, using genomic clones and cDNA clones isolated from a rat brain cDNA library. In contrast to the human gene, there is no alternative RNA processing observed in rat tissues expressing this gene. The coding regions for GRP and its associated peptide are evolutionarily conserved, consistent with a biologic function for both domains. The rat GRP gene is transcribed from a tissue specific promoter in the brain, creating an alternative route for regulating gene expression. The brain specific promoter is used only in selected nuclei in the rat brain when analyzed at the cellular level by *in situ* hybridization.

B. The human prepro GRP gene was successfully overexpressed in a novel baculovirus protein expression system. Using this model system, sufficient protein can be produced to allow analysis of post-translational processing events needed to generate biologically active peptides, and purify those peptides to establish function.

C. We have designed and synthesized consensus oligonucleotide probes specific for the receptor-binding biologically active carboxyl heptapeptide of bombesin family peptides. This oligonucleotide probe is currently being used to obtain and characterize other bombesin family genes expressed in neural tissue.

PROJECT DESCRIPTION

The Molecular Biology of the Mammalian GRP Gene Family

Professional Staff:

PI:	James F. Battey, MD, PhD	Senior Investigator	NCI-NMOB
Others:	Anne-Marie Lebacq-Verheyden, MD, PhD	Guest Researcher	NCI-NMOB
	Geoffrey Krystal, MD, PhD	Medical Staff Fellow	NCI-NMOB
	James Way	Microbiologist	NCI-NMOB

Collaborating Branches:

Kathleen Coelingh, Ph.D., Laboratory of Infectious Diseases, NIAID

Introduction:

Gastrin releasing peptide is a 27 amino acid neuropeptide representing the mammalian homologue to the amphibian mitogenic peptide bombesin. These two peptides share an identical carboxy-terminal, α-amidated heptapeptide, which is necessary and sufficient for binding to high-affinity cell surface receptors, initiating the physiologic responses observed in central and peripheral neurons, and in secretory cells. In addition, bombesin and GRP are potent mitogens for a number of cultured cells, including Swiss 3T3 mouse embryo fibroblasts, human bronchial epithelial cells, and small cell lung cancer cells. The latter cells both synthesize and secrete a biologically active bombesin like immunoreactive peptide, shown by molecular analysis to be the gene product of the prepro GRP gene. When a monoclonal antibody whose epitope overlaps the GRP carboxyl heptapeptide and prevents ligand binding to surface receptors, the growth of some SCLC cell lines is impaired both in a soft agar cloning assay and in nude mouse xenografts. These data suggest that under some circumstances GRP could stimulate the growth of the same cells which secrete it. Such an autocrine growth stimulation is thought to contribute to the acquisition of a malignant phenotype. In support of this hypothesis, both autocrine growth stimulation and tumorigenicity were induced in a factor dependent hematopoietic cell line given a highly expressed GM CSF growth factor gene. Further, rat fibroblasts expressing cell surface receptors for TGF-α showed more vigorous clonal growth in soft agar and increased tumorigenicity in nude mice after transfection with a constitutively expressed TGF-α gene. This malignant progression was reversible when antibodies to TGF-α were added to medium surrounding the cells. Our laboratory is currently undertaking a molecular analysis of the mammalian prepro GRP gene family (prepro GRP and prepro Neuromedin B) in an effort to both define its structure, regulation, and expression, as well as to explore the biologic properties of this gene in both normal and malignant cells.

Progress Report:

Expression of Rat prepro GRP Gene

We have cloned, structurally characterized and sequenced cDNA and genomic clones encoding the rat preproGRP gene. The gene is transcribed from two promoters, one of which is active only in the central nervous system. There is no evidence for alternative processing of the rat GRP gene, in contrast to the situation for the previously characterized human homologue. In situ hybridization studies show that a number of nuclei in the brain express GRP at high levels, while other areas are transcriptionally silent. Only a few selected GRP expressing nuclei use the brain-specific promoter, indicating that regulation of expression differs in different brain nuclei.

Regulation of the Human preproGRP Gene

Using cell lines which express preproGRP at high levels, we have established several features of the mechanism of regulation. The regulation occurs at the level of primary transcription, and is promoter mediated. A family of DNase hypersensitive sites are found only in the promoter region of expressed GRP genes. These chromatin structural changes often herald the existence of *cis* acting regulatory domains that bind *trans* acting transcription factors mediating both positive and negative gene regulation. Using both structural and functional assays, we are presently attempting to define more precisely the nature of this regulatory motif.

Post Translational Processing of the GRP Prohormone

To generate biologically active peptides from neuropeptide precursor prohormones, a complex series of cleavage and modification events must occur. In the case of proGRP, this includes cleavage at a pair of basic amino acids immediately following the twenty seven amino acid GRP peptide, and alpha-amidation of the terminal methionine residue. We have explored the regulation of these critical post-translational events, and have established that only a limited subset of neuroendocrine cells possess the necessary enzymatic machinery to perform these critical events successfully. More specifically, fibroblasts cannot efficiently cleave at the basic amino acid pair, and consequently secrete prohormone rather than processed, biologically active peptides. A moth ovary cell (Sf9) used to overexpress and process many eukaryotic proteins in conjunction with recombinant baculovirus infection cleaves near the appropriate site, but cannot amidate the GRP peptide resulting in a GRP peptide that is much less potent in a series of bioassay systems. Some human neuroendocrine SCLC cell lines efficiently cleave and amidate, to make biologically active GRP. Interestingly, these cell lines also cleave at several locations in the carboxyl extension peptide domain of proGRP, generating other peptides that potentially have biological activity. The bioactivity of these other peptides is currently under investigation.

Cloning Other Bombesin Family Genes

We have designed oligonucleotide probes that should recognize cDNA clones encoding the heptapeptide portion of bombesin GRP that is critical for biological activity. These probes could be used to clone other genes with a similar structural domain and spectrum of bioactivity to GRP, such as neuromedin B. A comprehensive rat brain cDNA library was constructed as a source for the clones, since most bombesin like peptides are expressed in the central nervous system. Using this approach, we have extracted several interesting candidate clones that may represent other bombesin family members. These clones are currently being analyzed in detail.

Publications:

1. Coelingh K, Murphy B, Collins P, Lebacq-Verheyden A, Battey J. Expression of biologically active and antigenically authentic parainfluenza type 3 virus hemagglutinin - neuraminidase glycoprotein by a recombinant baculovirus. Virology 1987;160:465-472.

2. Minna J, Battey JF, Brooks B, Cuttitta F, Gazdar A, Johnson B, Ihde D, Lebacq-Verheyden A, Mulshine J, Nau M, Oie H, Sausville E, Seifter E, Vinocour M. Molecular genetic analysis reveals chromosomal deletion, gene amplification, and autocrine growth factor production in the pathogenesis of human lung cancer. Cold Spring Harbor Symp Quant Biology, 1986;LI:843-853.

3. Lebacq-Verheyden AM, Segal S, Cuttitta F, Battey J. Swiss 3T3 mouse embryo fibroblasts transfected with a human prepro-GRP gene synthesize and secrete pro-GRP rather than GRP. J Cell Biochem 1988;36:237-248.

4. Lebacq-Verheyden AM, Markowitz S, Sausville E, Krystal G, Way J, Battey JF. Expression of the gastrin-releasing peptide gene in small cell lung cancer. Lung Cancer, in press.

5. Lebacq-Verheyden AM, Kasprzyk PG, Raum MG, Van Wyke Coelingh KL, Lebacq JA, Battey JF. Post-transcriptional processing of endogenous and of baculovirus-expressed human gastrin-releasing peptide precursor. Molec Cell Biol, in press.

6. Cuttitta F, Fedorko J, Gu J, Lebacq-Verheyden A, Linnoila R, Sausville E, Battey JF. Gastrin-releasing peptide gene associated peptides (GGAPs) are expressed in normal human fetal lung and small cell lung cancer: a novel peptide family found in man. J Clin Endo Metab, in press.

7. Battey JF, Lebacq-Verheyden AM, Krystal G, Markowitz S, Sartor O, Way J. Expression, regulation, and post-translational processing of the human prepro gastrin-releasing peptide. Ann NY Acad Sci, in press.

8. Lebacq-Verheyden AM, Krystal G, Sartor O, Way J, Battey JF. The rat prepro gastrin-releasing peptide gene is transcribed from two initiation sites in the brain. Molec Endo, in press.

9. Markowitz S, Krystal G, Lebacq-Verheyden AM, Way J, Sausville EA, Battey JF. Transcriptional activation and DNase I hypersensitive sites are associated with selective expression of the gastrin-releasing peptide gene. J Clin Invest, in press.

10. Krystal G, Birrer M, Way J, Nau M, Sausville E, Thompson C, Minna JD, Battey JF. Multiple mechanisms for transcriptional regulation of the myc gene family in small cell lung cancer. Molec Cell Biol, in press.

11. Krystal G, Way J, Battey JF. Comparison of c-, N-, and L-myc transcriptional regulation. In: Potter M, ed. Mechanisms of B-cell Neoplasia. New York: Springer-Verlag, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06579-05 NMOB

PERIOD COVERED
October 1, 1988 to September 30, 1989

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I.R. Kirsch, M.D.	Senior Investigator	NCI-NMOB
Others:	V. Bertness	Biol. Lab . Tech.	NCI-NMOB
	K. Nakahara	Biol. Lab. Tech.	NCI-NMOB
	M. Davey, M.D.	Staff Fellow	NCI-Metabolism

COOPERATING UNITS (if any)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology, and Immunology Section

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Association of a specific chromosomal abnormality wtih tumor type is well established and may reflect mechanisms of oncogenesis peculiar to that tumor. Alternatively, it may be that these associations reflect the particular differentiated state of the malignant cell, consistent with the model that rearrangements occur only within chromatin in an "active" configuration.

Our focus this year is on abnormalities of human chromosonome 14 in "normal" and malignant lymphocytes. Previously we indentified the formation of T-cell receptor (TCR) alpha chain immunoglobulin heavy (IgH) chain hybrids as a consequence of the inversion of chromosome 14 in both a T and B cell malignancy. This inversion appeared to be morphologically identical to the occasional inversion of this chromosmoe in the cells of all normal individuals. We have investigated the frequency of the normal occurance of this sporadic abnormality. We are using the polymerase chain reaction to develop a rapid means of assessing the molecular nature of these sporadic but consistent events.

A related but distinctive inversion or translocation of chromosome 14 is most likely associated with a particular type of T-cell malignancy, as well as with a clonal proliferation of T-cells in patients with the disease, ataxia-telangiectasia. We are cloning and characterizing the chromosome 14 breakpoints from T-cells from a patient with ataxia-telangiectasia and from a cell line derived from a patient with a particular T-cell lymphoma. The proximal breakpoints in both these cases is again (as above) within the TCR alpha locus. The distal breakpoints do not appear to involve IgH, but rather identify a more proximal region perhaps containing a new growth effecting gene function.

This research, utilizing our full complement of research tools, (cell culture, chromosome in situ , basic molecular biology) leads to the understanding of the fundamental issue of chromosome instability and its impact on oncogenes.

PROJECT DESCRIPTION

Chromosomal Abnormalities that Highlight Regions of Differentiated Activity

Professional Staff:

PI:	Ilan R. Kirsch, M.D.	Senior Investigator	NCI-NMOB
Others:	V. Bertness	Biol Lab Tech	NCI-NMOB
	K. Nakahara	Biol Lab Tech	NCI-NMOB
	M. Davey	Staff Fellow	Metabolism

Objectives:

Long Term

1. To define the necessary and/or sufficient features for chromosomal breakage and rejoining in different cell types.
2. To determine if chromosomal breakage disorders, particularly ataxia-telangiectasia, represent an exaggeration of normal events or a novel pathology.
3. To use the occurrence of cell-type specific chromosomal aberrations as an in-road to the exploration of differential gene activation during development.
4. To contribute to the understanding of how gene rearrangements mediated by chromosomal aberrations alter the regulation of the affected loci.

Short Term

To study chromosomal aberrations in hematopoietic cells, particularly:

1. To determine the frequency and cell type distribution of inversions and translocations of human chromosome 14 in normal, "premalignant", and malignant conditions and explore whether there is evidence for selective or random associations between particular breakpoints and particular transformed or proliferative states.
2. To clone and sequence the breakpoints from "normal" and malignant cells carrying inversions or translocations of chromosome 14. To assess from sequence data, if possible, how the chromosomal aberration occurred and what loci were involved.
3. To study the genomic activity of loci involved in translocations and inversion of chromosome 14 in corresponding cells in which the chromosomal aberration has or has not occurred, and thereby determine if the aberration has caused deregulation or altered expression of these loci.
4. To continue our studies on the mechanisms of oncogene deregulation as a result of chromosomal rearrangements in lymphoid malignancies.

Major Findings:

Previously (see previous annual reports) we have demonstrated that in hematopoietic cells, chromosomal abnormalities often involve regions of differentiated activity. Using this concept as a predictive and testable model we recently focused on chromosomal abnormalities associated with T cell malignancies, mapped the T cell antigen receptor genes by chromosomal *in situ* hybridization, and analyzed two cases of inversions of chromosome 14 that involved the T cell receptor alpha chain locus. These inversions had been formed by a site-specific recombination event between a T cell antigen receptor alpha chain joining (J) segment (at 14q11.2) and an immunoglobulin heavy chain variable (V) segment (at 14q32.3). The formation of the hybrid gene, part immunoglobulin, part T cell receptor, provided an attractive model for the generation of lymphoid malignancies. Immunoglobulin (Ig) and T cell receptors (TCR) are, after all, cell surface receptors capable of transducing mitogenic (antigenic) stimuli at the cell surface into cellular proliferative activity. It was conceivable that either because of its function or specificity a hybrid Ig/TCR gene might stimulate an abnormal proliferative response. Observations in certain retroviral transduction systems lent support to this view. Occasionally a retrovirus containing an intact antigen receptor gene can be found associated with certain types of lymphoid malignancies.

While still an attractive model our further studies suggest that the contribution of translocation and/or inversion of chromosome 14 to the development of lymphoid malignancy is more complicated than it first appeared. We have found that the natural occurrence of inversions of chromosome 14 is rather high, 0.01-0.1%. Furthermore, in the lymphoid malignancies which show the clearest association of a translocation or inversion of chromosome 14 the 14q32 chromosomal breakpoint does not seem to involve the Ig heavy chain locus as it had in the two sporadic tumors we had analyzed above.

We are analyzing in molecular detail a t(14;14) found in a cell line derived from a patient with a T cell leukemia. The proximal breakpoint is clearly within the TCR alpha chain joining region. The distal (14q32) breakpoint involves a region 3' and centromeric to the IgH locus. Further insight into this issue may be gained from our studies of material from patients with the disease, ataxia-telangiectasia, AT. AT is a disease of protean manifestations including progressive neurologic deterioration, ocular telangiectasia, immunodeficiency, a tendency to develop chromosomal breaks, and a predisposition to cancer, particularly lymphoid malignancies. The karyotype of PHA stimulated lymphocytes from patients with AT will often show translocations and inversions within and between chromosomes 7 and 14 involving the chromosomal bands to which are localized the TCR alpha beta, gamma, and delta chain. This underscores the fact that in T cells chromosome abnormalities do indeed often involve regions of differentiated activity and highlight the propensity of these patients to develop cell-type specific chromosomal breaks. This may be a unique defect in this disease or an exaggeration of a normal error-prone lymphoid recombination system. This distinction is being investigated in our laboratory at present. Occasionally a lymphoid cell is generated in patients with AT that seems to have a proliferative advantage. These cells almost always carry inversions or translocations of chromosome 14. They can reach a point where they comprise

100% of the PHA stimulatable population in the patient and can be shown by Southern blot to represent a single T cell clone. We have identified and analyzed one such t(14;14) from a 32 year old female with AT. Analogous to the T-ALL cell line the proximal breakpoint in this patient's cells was again within the TCR alpha chain J region, the distal breakpoint within a region 3' and centromeric to the IgH locus. The two 14q32 breakpoints analyzed in these two cases have not yet been linked to each other, nor has a new presumptively growth effecting gene yet been identified in this region. We feel these studies are addressing fundamental issue in multi-step carcinogenesis and genomic instability. We have recently started to analyze two related chromosomal aberrations which share one or the other region with these t(14;14) cases. One is a lymphoid/myeloid cell line carrying a t(1;14) (p32;q11.2) in which there is clear rearrangement of the TCR alpha and delta loci. The other is a tumor sample from a patient with ALL whose tumor cells appear to carry a t(5;14) (q33;q32). We are focusing on the basic contribution of these loci to the development of lymphoid/myeloid malignancy and the necessary and sufficient factors required for the mechanism of chromosomal breakage and regioning to operate.

Publications:

Hecht F, Hecht BK, Kirsch IR. Fragile sites limited to lymphocytes: Molecular recombination and malignancy. Cancer Genet Cytogenet 1987;26:95-104.

Lipford E, Wright JJ, Urba W, Whang-Peng J, Kirsch IR, Raffeld M, Cossman J, Gongo DL, Bakhshi A, Korsmeyer SJ. Refinement of lymphoma cytogenetics by the chromosome 18q21 major breakpoint region. Blood 1987;70:1816-1823.

Hollis GF, Gazdar AF, Bertness V, Kirsch IR. A complex translocation disrupts c-myc regulation in a human plasma cell myeloma. Molec Cell Biol 1988;8:124-129.

Denny CT, Hollis GF, Kirsch IR. Germline immunoglobulin VH regions and transcribed in T cells. Submitted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06581-05 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Differentiation and Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Timothy Bender, PhD (term 12/31/88)	Senior Staff Fellow	NCI-NMOB
	Shoshana Segal, PhD	Independent Invest, USUHS	NCI-NMOB
	Diana McClinton	Microbiologist	NCI-NMOB
	Cynthia Timblin, PhD (EOD 1//17/88)	Staff Fellow	NCI-NMOB
	Francine Foss, MD (EOD 5/1/88)	Medical Staff Fellow	NCI-NMOB
	Lillian Raveh, PhD (EOD 8/2/87)	Guest Researcher	NCI-NMOB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Molecular Biology of Differentiation

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5.5	5.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Overall Objectives:

1. To clarify the cellular and molecular mechanisms which determine and regulate hematopoietic and stem cell differentiation.

2. To clarify the relationship between differentiation and malignancy.

B. Species Studied: Mice and humans

We have been studying the role of nuclear oncogenes in growth and differentiation. Much of our work has focused on c-*myb* which encodes a nuclear, DNA-binding protein. We find that the major mechanism by which c-*myb* expression is regulated in mouse hematopoietic cells is modulation of transcription elongation occurring at a site in intron 1. Preliminary results suggest a similar kind of regulation of c-*myb* in human cells. We have demonstrated that pre-B cell lines express 10-100 times as much c-*myb* mRNA as more mature B cells. This finding has helped us to demonstrate that pre-B cell lines which begin to express surface immunoglobulin retain a pre-B cell phenotype. In addition, we have established that down-regulation of c-*myc* mRNA is necessary for mouse erythroleukemia cells to undergo terminal differentiation. Results obtained recently suggest that down-regulation of c-*myb* mRNA is also necessary for terminal differentiation. These studies suggest that constitutive expression of normal proto-oncogenes may account for the observation that tumor cells are generally "frozen" in a particular state of differentiation.

PROJECT DESCRIPTION

Molecular Genetics of Differentiation and Transformation

A. Structure, expression, and function of murine c-myb proto-oncogene

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Timothy Bender, PhD (term 12/31/88)	Senior Staff Fellow	NCI-NMOB
	Diana McClinton	Microbiologist	NCI-NMOB

Three kinds of approaches have been used (i.e., tumor cell lines, induction of differentiation in a cloned cell line, and somatic cell hybrids) to study differential expression of c-myb proto-oncogene mRNA in murine B cell tumors. To summarize, in murine B-cell tumor lines and hybrids, the level of c-myb mRNA expression reflects the level of B-cell differentiation. The results obtained for the line that differentiates are also consistent with the concept that c-myb expression is markedly down-regulated (ten-fold or more) as B cells mature beyond the pre-B cell stage of development. Since all studies were done with exponentially growing cells, the down-regulation of c-myb clearly correlates with differentiation and not with changes in cell proliferation.

We have determined the DNA sequence of murine c-myb mRNA. Starting with an avian V-myb probe we initially isolated genomic clones covering 30 Kb, including sequences at each end of this span which hybridize to c-myb mRNA but not to a V-myb probe. We prepared a murine pre-B cell cDNA library, and isolated ten c-myb cDNA clones. Together two of our cDNA clones include approximately 3.4 Kb of c-myb mRNA sequence. The sequence of these clones, together with S1 nuclease protection studies, have defined the murine c-myb transcription unit. In the course of these studies we have found an extreme degree of heterogeneity of the 5′ end of murine c-myb mRNA. We are attempting to clarify the biological significance of this finding. We have begun to extend our studies to the human c-myb gene. To assess potential 5′ regulatory sequences we sequenced the 5′ end of the coding region plus about 1.5 Kb of flanking region of human c-myb genomic clones; there is striking homology to the mouse gene even in 5′ flanking sequences. Recently we have demonstrated that a major mechanism regulating the level of c-myb mRNA expression in murine B cell lines is a block to transcription elongation (? pausing or ? premature termination) occurring near the middle of intron one, a genomic region which contains a more predominant DNaseI hypersensitive site in a B cell line (A20/2J) compared to a pre-B cell line (70Z/3B). We are also studying the structure and expression of the c-myb gene in a small cell lung cancer line which has amplified the c-myb gene. Finally we are beginning to: 1) express c-myb in yeast in order to prepare antisera and to provide large amounts of c-myb protein for study, 2) transfect c-myb expression vectors into various eukaryotic cells, and 3) look for c-myb gene rearrangements and amplification in other human tumors. Our long term goal is to determine the role of the c-myb proto-oncogenes in hematopoietic differentiation and tumor induction.

Publications:

1. Bender TP, Kuehl WM. Structure and differential regulation of the myb proto-oncogene in murine B lymphoid tumors. In: UCLA Symposia on Molecular and Cellular Biology, eds. Cellular and Molecular Biology of Tumor and Potential Clinical Applications. New York: Alan R Liss, Inc, 1986;56:239-246.

2. Bender TP, Kuehl WM. Differential expression of the c-myb proto-oncogene marks the pre-B/B cell junction in murine B lymphoid tumors. J Immunol 1987;139:3822-3827.

3. Bender TP, Thompson C, Kuehl WM. A block to elongation in the first intron of c-myb is responsible for regulating transcription levels in murine B lymphocytes. Science 1987;237:1473-1476.

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B. Role of c-myc and other oncogenes in differentiation (in collaboration with M. Birrer)

Professional Staff:

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
PI:	Shoshana Segal, PhD	Independent Invest., USUHS	NCI-NMOB
Others:	Timothy Bender, PhD	Senior Staff Fellow	NCI-NMOB
	Diana McClinton	Microbiologist	NCI-NMOB
	Francine Foss, MD (EOD 5/1/88)	Medical Staff Fellow	NCI-NMOB
	Lillian Raveh, PhD (EOD 8/2/88)	Guest Researcher	NCI-NMOB

Endogenous c-myc and c-myb mRNA levels decrease rapidly when murine erythroleukemic (MEL) cells are induced to differentiate with DMSO. We have introduced a c-myc construct into MEL cells by stable transformation in order to determine whether constitutive expression of myc mRNA blocks differentiation or inhibits expression of endogenous myc mRNA. The expression of exogenous human c-myc mRNA DMSO-induced differentiation of MEL cells. In addition, expression of endogenous c-myc mRNA is not turned off by exogenous c-myc, although the level of expression of the endogenous c-myc is inversely proportional to the level of expression of exogenous c-myc. Preliminary results suggest that expression of high levels of human L-myc mRNA from a transfected expression vector also blocks inducer mediated MEL differentiation, even though endogenous L-myc mRNA is not detected in MEL cells. We also find that MEL cells which constitutively express an exogenous c-myb construct are blocked in their ability to terminally differentiate when an appropriate induced is added. In addition, studies in F-9 teratocarcinoma cells indicate that the block of differentiation by constitutive c-myc expression is general. Studies are underway to determine the mechanisms which cause down-regulation of c-myc mRNA during F-9 differentiation.

Publications:

1. Kuehl WM, Bender TP, Stafford J, McClinton D, Segal S, Dmitrovsky E. Expression and function of the c-myb oncogene during hematopoietic differentiation. Current Topics Microbiol Immunol, in press.

2. Lebacq-Verheyden AM, Segal S, Cuttitta F, Battey JF. Swiss 3T3 mouse embryo fibroblasts transfected with a human prepro-GRP gene synthesize and secrete pro-GRP rather than GRP. J Cell Biochem 1988;36:237-248.

3. Birrer MJ, Segal S, DeGreve JS, Kaye F, Sausville EA, Minna JD. L-myc cooperates with ras to transform primary rat embryo fibroblasts. Mol Cell Biol 1988;8:2668-2673.

4. Segal S, Dmitrovsky E, Paez de la Cadena M. The effect of a transfected c-myc proto-oncogene on cellular differentiation. Mol Immunol, in press.

C. Identification of genes uniquely expressed in mouse plasmacytoma cells

PI:	W. Michael Kuehl, MD	Senior Investigator	NCI-NMOB
Others:	Cynthia Timblin, PhD (EOD 1/17/88)	Staff Fellow	NCI-NMOB

Mouse plasmacytoma cell lines differ from mature murine B cell lines that secrete immunoglobulin by several notable features: 1) morphology, 2) lack of expression of certain B cell markers (such as surface Ia antigen), 3) lack of endogenous c-*myc* mRNA expression, and 4) the consistent dominance of the plasmacytoma phenotype in somatic cell hybrids formed with all other types of lymphoid cells. We have recently begun to isolate cDNA clones which are expressed in mouse plasmacytoma cell lines but not in mature B cell lines. Our long term goal is to identify and isolate genes which both determine and contribute uniquely to the phenotype of terminally differentiated plasma cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06587-04 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Tumor-Specific Gene Alteration and Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	Carolyn Felix, MD	Medical Staff Fellow	NCI-NMOB
	Nita Seibel, MD (part-time)	Assistant Professor	NCI-NMOB

COOPERATING UNITS *(if any)*

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Genetics, Molecular Biology & Immunology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

Differential structural alterations and expression of immunoglobulin (Ig), T cell receptor (TCR), and various growth effecting genes are studied in malignant tumors and their derivative cell types. Studies are carried out to diagnose, classify, and stage lymphoid malignancies via a) Southern and Northern blot analysis and b) RNA-RNA tissue in situ hybridization.

A. DNA and RNA is extracted from the tumors of patients with acute lymphoblastic leukemia (ALL) of infancy, pre B ALL, T cell ALL, mycosis fungoides, and Sezary syndrome. The structural reconfigurations of DNA around the Ig and TCR loci resulting from the normal functional activation of these loci in these cells provide unique "fingerprints" for identifying clonal populations in the samples and following these populations during the course of treatment. We have shown by these analyses that the above listed lymphoid malignancies each manifest generally distinguishing genotypic patterns reflecting target cell maturation which to a certain extent recapitulates the age incidence of the development of these tumors. These studies are now being extended in an exhaustive analysis of 90 patients with B cell precurser ALL. Correlations between genetype and clinical presentation and cause are being established. In addition, we are establishing criteria for the definitive genotypic diagnosis of particular tumors.

B. RNA-RNA tissue in situ hybridization. The expression of individual cells within tissue sections from lymph node biopsies and peripheral blood from patients with lymphoid malignancies have been analyzed with immunoglobulin, T cell receptors, and oncogene probes. This technique refines the analysis of such tissue to the point where the unique gene expression of one cell in hundreds of thousands can be identified. Futhermore we have developed a technique of direct RNA sequencing the allows us to utilize tumor specific gene expression as a tumor specific marker providing a potentially powerful and sensitive means of cancer diagnosis and staging.

PROJECT DESCRIPTION

Tumor Specific Gene Alteration and Expression

Professional Staff:

PI:	Ilan R. Kirsch, MD	Senior Investigator	NCI-NMOB
Others:	Carolyn Felix, MD	Biotech. Fellow	NCI-NMOB
	Virginia Bertness	Biol Lab Tech	NCI-NMOB
	Kenny Nakahara	Biol Lab Tech	NCI-NMOB
	Nita Seibel, MD	Guest Researcher	NCI-NMOB

Objectives:

1. To develop, master, and refine techniques based on molecular genetics which are of direct current application in the diagnosis, classification, and staging of patients with cancer.

2. To demonstrate the usefulness of these techniques in pilot studies.

3. To promote the adoption of these techniques by service oriented laboratories, and supervise the implementation of such techniques in a standardized quality controlled fashion for comprehensive, prospective, best available therapy protocols and epidemiological studies.

4. To use the data generated in the pilot and/or comprehensive analyses as a resource of information and a bank of material for further studies to be carried out in basic research laboratories.

Methods Employed:

As a result of the normal functionally activating recombination events that occur in B and T lymphocytes, the structural configuration of the DNA around the immunoglobulin or T cell receptor loci is irreversibly altered in each differentiated cell. Because of the unique nature of the antigen-receptor molecules being produced, the VJ or VDJ recombinatorial event (and DNA configuration) in one cell making one antibody or T cell receptor will be distinct from a cell making an antibody or T cell receptor with a different antigen-binding capacity. Thus, by the necessity of its role in the immune response, every lymphocyte beyond a certain stage in its development carries within it a unique molecular DNA "fingerprint".

A B or T lymphocytic malignancy results in the clonal proliferation of a cell that therefore can carry a unique molecular fingerprint. Recombinant DNA technology now allows the recognition of this fingerprint when it is present in 0.1% to 1% of a total cell population. Therefore, molecular biology can increase the sensitivity of tumor detection in a tissue sample. It can classify tumors as being of T or B cell lineage, identify biclonal tumor populations, distinguish new primary lymphomas from relapsed cases, and address basic questions of tumor development and progression. This basic molecular technology can now be successfully combined with fine needle aspiration of lymph nodes to aid in the diagnosis and staging of patients with lymphomas. It may very likely become a routine test in the general work-up of patients with lymphoma at presentation and at relapse. There is an essential need to incorporate the information that will be generated from these tests into prospective clinical trials. After these trials it can be anticipated that routine management of patients with lymphoma will be influenced by molecular data as well as the other currently available analyses.

This technology is not restricted to use in the leukemias and lymphomas. Although the normal functional rearrangements that these cells undergo provide a special feature for studying lymphoid malignancies the molecular biological techniques are of general use. It is possible that gene systems in other cell types will undergo similar DNA configuration-altering recombination events as recombination signals exist outside of the immunoglobulin loci themselves, possibly in other gene systems. Of more likely usefulness, however, will be the ability to monitor DNA rearrangements and amplifications that are directly related to the development of a cancer and not just markers of the transformed cell type. This is a likely possibility as more and more specific cancers are being shown to be associated with distinctive chromosomal aberrations: translocations, amplifications, deletions, or inversions. On the level of the DNA, these aberrations often result in the development of distinguishing new configurations of DNA (a tumor-specific molecular fingerprint) often related to oncogenes; i.e. genes that are thought capable of contributing to the development of a malignant phenotype in a normal cell. Thus, a wide variety of tumors are approachable using the same basic technology. In many cases these chromosomal aberrations contribute to the etiology of cancers by altering the quality of quantity of RNA transcribed and translated from those genes structurally flanking the chromosomal aberrations. It is now possible to identify (at the level of individual cells within histologic tissue sections) those cells that are expressing a particular gene or genes. This technique is called RNA-RNA tissue in situ hybridization. Thus, technically, the potential exists to redefine histopathologic diagnoses on the basis of particular gene expression within tumor cells.

Recently a new technique, "polymerase chain reaction" or "PCR", has been developed that in certain conditions allows the identification of genomic rearrangements present in tumor samples at much less than 0.1% and also greatly increases the ability to identify particular mRNAs (or their corresponding cDNAs) of interest.

Over the next 5 years pathologists may begin reporting information about rearrangements, amplifications, and aberrant expression of various oncogenes in tumor biopsy specimens which an oncologist will then use to define prognosis or design a regimen of therapy. For the practicing oncologist, the molecular causes and consequences of a patient´s disease will become relevant to determining that patient´s diagnosis, course, and treatment.

Major Findings:

Patient Studies

The ability to detect immunoglobulin (Ig) and T cell antigen receptor (TCR) gene rearrangements is proving useful in confirming diagnosis of suspected B or T cell lymphoid malignancies and in establishing mono, oligo, and polyclonality in these and possibly related disorders.

Mycosis Fungoides, Sezary Syndrome, T-Gamma Lymphocytosis

In an early study we employed cloned probes for the TCR loci and Southern blot analysis (see references to basic molecular biological techniques at the end of reference list) to determine whether gene rearrangements were present in human T cell neoplasms representing various stages of T cell development. Gene rearrangements were present in all cases of immunophenotypic T cell disorders except a single case of T gamma lymphocytosis. Subsequent work by us and others have shown this disorder to be variable in terms of identification of clonal TCR rearrangements possibly reflecting disease heterogeneity and/or temporal evolution of clonality during the disease process. Germline gene configurations were present in all patients with "mature" non T cell neoplasms and in uninvolved tissue from patients with T cell lymphoid.

B Cell Precursor ALL of Childhood

An exhaustive study of over 80 patients seen at the NIH and within the Children's Cancer Study Group (CCSG) affiliated hospitals is being completed. The DNA will have been analyzed with Ig heavy, kappa, and lambda probes and TCR alpha, beta, gamma, and delta correlations with clinical course and presentation will be established. The requisite workup for a complete genotypic study will be defined.

Acute T Cell ALL of Childhood

TCR and Ig genes were examined in 26 cases of childhood T-ALL and 17 cases of pre B ALL. TCR gamma was rearranged in 22 of the 26, TCR beta in 23 of the 26, one or the other or both in almost every case of phenotypic T-ALL. Rearrangement of both alleles of the gamma and beta chains occurred in most T-ALLs. Expression of the beta and/or gamma chains was observed commonly in these tumors. Alpha chain gene expression was found less often, usually only in the most mature T-ALL's which were T3+. Analogous to studies of pre-B ALL molecular analysis of T-ALL suggests a hierarchy of early gamma and beta gene rearrangement followed by alpha, and a coordinate sequence of early appearance of the 3A1 antigen, followed by T11 and later T3. Three patients in this series with T-ALL also showed evidence of Ig heavy chain gene rearrangement. Of the 17 patients with pre-B ALL eight showed rearrangements of the TCR gamma gene. Evidence of activity of these two loci, felt to encode distinct differentiated functions, is much more commonly seen in these tumors of lymphocytes of less mature stages of development than in the more mature adult tumors such as those mentioned earlier. This may speak to an interesting issue of lymphocyte development which has been addressed in a separate project report.

Acute ALL of Infancy

ALL in infants less than one year of age runs a particularly virulent course. It, therefore, was of interest to geneotypically analyze a group of these patients to possibly contrast their patterns of gene rearrangement with those seen in older children as described above, as well as those seen in adults (See

Table 1. GENOTYPES AND PHENOTYPES OF INFANT ALLS

Pt.#	Ig H-Chain		k L-Chain		TCR	TCR	HLA-DR	B4	CALLA	SIg	3A1	T11	L4F3	Ig10/ 5FI	13.1	C7E10	12-8
1	R	1	G		G	G	+	+	-	-	-	-	-	-	-	-	17
2	R D	1 1	G		G	G	+	+	-	-	-	-	-	-	-	-	-
3	R	2	G		G	G	+	+	15	-	-	-	-	-	-	-	-
4	G		G		G	G	+	+	15	-	-	-	-	-	-	-	-
5	R	2	R	1	G	G	-	32	36	-	-	-	-	-	-	20	-
6	G		G		G	G	+	+	-	-	-	-	12	-	-	-	-
7	R	1	R	1	G	R 1	+	+	11	-	-	-	-	-	-	-	-
8	G		G		G	G	+	+	-	-	-	-	-	-	-	-	-
9	R	1	G		G	G	+	+	-	-	-	-	-	-	-	-	-
10	R	2	G		G	G	+	+	-	-	-	-	-	-	-	-	-
11	G		G		G	G	+	+	-	-	-	-	-	-	-	-	-

Legend: Pt. = Patient, R = Rearranged, D = Deleted, G = Germline, No. = Number of alleles, (-) is <10% of cells showing reactivity, (+) is >50%, and specific values (%) are given for the 10-50% range. Phenotypic data (except for SIg and 3A1) were previously tabulated in Dinndorf and Reaman. The germline configuration of the Ig H-chain locus was confirmed for patients 4 and 11 using HindIII and/or EcoRI digests in addition to BamHI digests probed with the 3′J_H probe. The germline configuration of the γTCR locus was found in every case by probing BamHI digested with DNA with the $C_T\gamma$, $J\gamma_{1.3}$, and an entire TCRγcDNA, and further confirmed by hybridization of EcoRI blots with the $J\gamma_{1.3}$ probe for patients 4, 5, 10, and 11. The β TCR locus was studied by hybridization of BamHI digested DNA with the $C_T\beta$ probe in all cases, and hybridization of EcoRI digested DNA with the same probe for patients, 4, 5, 10, and 11.

Table 1). Lymphoblasts of 11 infants demonstrated surface antigens which have been correlated with a pre-B cell phenotype. 4/11 of these retained the germline configuration of both Ig and TCR genes, suggesting that ALL in infancy represents an earlier stage of B cell development than is found in pre-B ALL of older children, where all had rearranged at least Ig H-chain genes. No phenotypic T-ALL patients were found in this study. As mentioned above, Ig H-chain rearrangements were occasionally seen in the series of T-ALL's, and TCR gene rearrangements, especially gamma, were identified in a large proportion (45%) of pre-B ALL's of older children. In contrast, TCR gamma gene rearrangements were not identified in pre-B ALL's of infants. These studies extend the earlier studies of pre-B ALL in other children. Together they suggest discrete stages in lymphoid development vulnerable to malignant transformation. Because the distribution of ALL subtypes throughout childhood is not uniform, these data suggest either a change in size of the target cell population, or a differential vulnerability of lymphocyte subpopulations to etiologic agents with increasing age.

Family Studies

Insertions, deletions, amplifications, and point mutations of genes occur in DNA throughout the evolutionary process. When these events occur in germline DNA, they are transmitted vertically from one generation to the next. Differing mutation sites and mutation rates between species and within a given species can be identified by comparison of defined populations by restriction enzyme site patterns for given probes of interest. This study of "restriction fragment length polymorphisms" ("RFLPs") reflecting the structural variability of DNA can be utilized in evolutionary, population, and family studies. Unlike the rearrangements of Ig or TCR genes, these RFLPs need not indicate the programmed rearrangement of a particular locus during the ontogeny of a particular cell type. Molecular genetic technology of DNA analysis can still be applied to this study. In what follows we describe one such example of RFLP analysis in which we have been involved. Its significance to the occurrence of a particular tumor in a particular family remains to be seen. Conceivably it could provide a marker for disease risk in this case. It would also be suggesting a mechanism of tumor formation akin to those now postulated, for example, for Wilm's tumor or retinoblastoma. We investigated a family in which a father and three of his offspring had meningioma with clinical onset at the ages of 35 to 65 years. A fourth offspring died of multiple neoplasms arising at 29 years. No one in the family had any evidence of von Recklinghausen disease. The three siblings with meningioma carried a constitutional Robertsonian translocation, t(14;22) (14qter cen 22qter), in peripheral blood leukocytes. Three other members of the second generation who were beyond the age at which the onset of meningioma is expected had no tumors and had normal karyotypes. In the third generation, whose members are now reaching the age for tumor onset, four carriers of the translocation have been identified; to date they are all asymptomatic except for one woman, who has breast cancer.

Both living members with meningiomas had a polymorphic variant of the c-sis oncogene in peripheral-leukocyte DNA, according to analysis with the Southern blot technique. This variation was also present in one asymptomatic member of the third generation and segregates with the morphologically normal No. 22 chromosome in both the affected and nonaffected members. It is possible that an "active" or mutant gene on the long arm of chromosome 22, possibly even the c-sis oncogene itself may be associated with the development of meningiomas in this kindred. Analysis of the meningioma tumor tissue itself would be of great interest in this regard but is presently not available. Sporadic meningioma is known to often to be associated with monosomy of chromosome 22 or less frequently the absence of the long arm of chromosome 22. The c-sis proto-oncogene has been localized to the tip of the long arm of chromosome 22.

Epidemiological Studies

We carried out a pilot feasibility study in collaboration with Dr. Ian Magrath of the Pediatric Branch, National Cancer Institute, USA and Dr. Gregory O´Conor, then Senior Scientist, International Agency for Research on Cancer, Lyon, France, to determine whether the logistics of tissue collection from widely dispersed and varied patient care centers could be set up so as to allow for appropriate and reproducible analysis of DNA rearrangements.

The most basic questions remain unanswered concerning worldwide presentation and distribution of different types of lymphoid leukemia and lymphoma. The initial screening assay on the patient samples was a determination of TCR beta and Ig heavy chain gene rearrangements. Samples were collected from Nagoya and Kyoto, Japan, from Riyadh, Saudi Arabia, from Lima, Peru, from two centers in Budapest, Hungary, and from New Delhi, India and sent to us via Lyon, France. We were easily able to study rearrangements of Ig and TCR loci in most of the samples received. Thus, it is reasonable to consider such international molecular genetic epidemiological studies. For a selected number of samples we extended our analysis to indicate studies of kappa and lambda light chain gene rearrangement and rearrangement of a select number of proto-oncogenes.

The ultimate goal of such an epidemiologic study would be to correlate the specific type of lymphoma with geographical distribution. In its most fundamental form this would involve relating the frequency of a specific Ig or TCR gene rearrangement and state of maturity of the tumor cell to the distribution of these malignancies in different parts of the world. Ultimately, the survey would be expanded to include information on oncogene expression and association with specific viruses. One need only consider how the recognition of endemic Burkitt´s lymphoma in equatorial Africa or HTLV-1 positive acute T cell leukemia in the southern provinces of Japan or the Caribbean has been of such crucial importance for basic research as well as patient diagnosis and treatment. Often on the basis of DNA analysis alone one is able to make significant progress toward patient diagnosis and classification.

On a sample of lymph node from Riyadh, Saudi Arabia for which no clinical or laboratory data were available, we were able to discern a rearranged Ig heavy chain gene and kappa light cahin gene, germline Ig lambda, TCR beta, and c-myc genes and a rearranged bcl-1 gene. On the basis of this analysis alone, it would be highly likely that such a patient suffered from a B cell follicular lymphoma with a t(14;18) translocation.

RNA-RNA Tissue In Situ Hybridization

As described in the previous section (as well as in the work of dozens of laboratories all over the world) multiparameter studies including cytochemistry, ultrastructural analysis, immunophenotyping, and recombinant DNA analyses of nucleic acids have contributed greatly in recent years to the classification and subclassification of lymphoid malignancies and other tumors. The kinds of recombinant DNA analyses described above examine the entire cell population, and thus the data represent an average of gene rearrangement or expression in which heterogeneity in the tumor can only be inferred. Recently it has become possible to use cloned nucleic acid probes at the level of individual cells in tissue sections. In conjunction with routine histologic diagnosis, this technique holds the promise of directly assessing cellular heterogeneity in a tumor, classifying tumor by their unique patterns of gene expression, and potentially gaining insight into etiologic and developmental questions in the disease state being analyzed.

As an early step in our utilization of this technique, we wished to establish the credibility of the data we would generate with it, with reference to the conventional techniques of molecular genetic analysis with which we were familiar and comfortable. We, therefore, conducted an analysis of an involved lymph node and peripheral blood from a patient with lymphadenopathy and a elevated white count. DNA and RNA from these tissues were analyzed by Southern and Northern blot, respectively, using ^{32}P probes derived from the Ig heavy and light and TCR beta chain loci. ^{35}S complementary RNA probes from these same loci were applied to fixed sections of these tissues and (after appropriate hybridization and washing conditions) autoradiographed.

We found the different techniques to be confirmatory and complementary. The technique is also consistent with immunophenotypic data generated on the same tissue. Our studies demonstrate that this patient suffered from a B cell malignancy. His lymph node showed a monoclonal B cell population and a polyclonal T-cell infiltrate. In his peripheral blood only the monoclonal B cell population was seen. Furthermore, in the malignant B cell population a provocative cellular heterogeneity was noted, a heterogeneity that showed an interesting distribution pattern within the lymph node studied. There was a diffuse clonal infiltrate of B lymphocytes expressing Ig mu and lambda message. Clustered around the vessels, however, were encountered apparently the same clonal population but with a much increased expression of the Ig mRNAs. The data and observations derived from this study supported our expectations that, when combined with clinical observations and other methods in histopathology, this kind of analysis could provide a general tool for answering important diagnostic and prognostic questions.

With this in mind we applied this technique to a study of *myc* related (c-*myc*, N-*myc*, L-*myc*) proto-oncogene expression in small cell lung cancer. The tissues investigated included cytospins of ten cell lines derived from patients with SCLC, four corresponding nude mouse xenografts from cell lines, and metastatic tumor tissue obtained by surgical biopsy and at autopsy. The expression of each gene was specifically demonstrated by autoradiography in the cytoplasm of the neoplastic cell samples. The average levels of oncogene expression in each specimen corroborated previous data obtained by Northern blot assay. In addition, heterogeneity in gene expression from cell to cell in each sample was noted. This study represented the first attempt to demonstrate oncogene expression in lung cancer cells *in situ*, and confirmed that the expression of these *myc* related genes can be seen in the primary tumor.

Chromosome In Situ Hybridization

Our group was one of those early involved in the localization of genes in the human genome by the technique of chromosome *in situ* hybridization following the technique first developed by Harper and Saunders. Since our first effort localizing the Ig heavy chain locus to 14q32, we have conducted numerous other studies. These studies have included the localization of c-*myc* to 8q24, the localization of the beta globin cluster to 11p15 (in collaboration with Dr. Cynthia Morton) the localization of L-*myc* to 1p32 and of an amplified c-*myc* to an HSR in a small cell lung cancer cell line (in collaboration with Dr. John Minna and Dr. June Biedler). We have localized the human beta 1-4 galactosyltransferase to 9p13 (within the same chromosome band as is found in the gene defective in the disease galactosemia). In collaboration with Dr. Tak Mak we localized the gene for the TCR alpha chain locus to 14q11.2. In collaboration with Dr. James Battey we localized the gene for human gastrin releasing peptide to 18q21. This was the same site to which we, in collaboration with Dr. Stan Korsmeyer, and other, had localized the bcl-2 gene. We have also utilized this technique as part of our molecular genetic analyses of the human T cell lymphoma line SUP-T1, a human myeloma cell line H929 and two patient samples carrying a t(14;14) (discussed in a separate report).

Proposed Course:

Our laboratory has established itself as having expertise in the basic techniques of DNA and RNA isolation and analysis, RNA-RNA tissue *in situ* hybridization, and chromosome *in situ* hybridization. Our specific aims and future plans fall into two categories, those that would be undertaken in-house primarily by the limited personnel of our own research laboratory, and those in which we would wish to participate as part of a much larger comprehensive study.

I. RNA-RNA Tissue In Situ Hybridization Studies

A. Analyses of testicular biopsy specimens for evidence of tumor cell infiltrate, at presentation or relapse in children with acute lymphoblastic leukemia.

B. 1. Direct RNA sequencing of Ig or TCR variable regions from leukemic cells. Synthesis of complementary oligonucleotide probes and the use of these probe in tissue *in situ* hybridization as tumor specific markers for clinical staging.

2. Direct RNA sequencing of mutable regions of expressed oncogenes from specific lymphoid and solid tumors. Depending on the extent of mutation, synthesis of complementary oligonucleotides probes for use as tumor specific markers as in B1.

II. Tumor Genotyping

We intend to continue our exploration of the utility and necessary and sufficient procedures for comprehensive genotyping of selected patient populations. For example correlations between genotypic data and clinical presentation and course of children with ALL will continue to be exhaustively pursued.

III. Tumor Genotyping Service

The establishment of a service facility for processing of tissue samples, and DNA and RNA preparation. The capacity to comprehensively screen selected patient populations or tumor samples for the rearrangement and/or expression of particular genes of interest. We are interested in maintaining a supervisory and quality control role over the activities of this laboratory. Envisioned is a NIH wide facility with a board composed of members from different institutes meeting to decide what questions should be addressed and to review the data being generated.

Publications:

1. Felix CA, Reaman GH, Korsmeyer SJ, Hollis GF, Dinndorf PA, Wright JJ, Kirsch IR. Immunoglobulin and T cell recpetor gene configuration in acute lymphoblastic leukemia of infancy. Blood 1987;70:536-541.

2. Colamenici OR, Ang S, Quinones R, Henkart P, Heikkila R, Gress R, Felix C, Kirsch I, Longo D, Marti G, Seidman JG, Neckers LM. Il-2 dependent expansion of CD3 + large granular lymphocytes expressing T cell receptor. J Immunol 1988;140:2527-2533.

3. Gu J, Linnoila I, Gazdar AF, Minna JD, Brooks BJ, Seibel N, Hollis GF, Kirsch IR. A study of myc related expression in small cell lung cancer by in situ hybridization. Am J Path, in press.

4. Kirsch IR. Molecular biology of the leukemias. Pediatric Clinics of North America, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06589-04 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology and in vitro Growth and Drug Sensitivity Testing of Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
Others:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
	Daniel Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
	Barnett Kramer, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Chief	NCI-NMOB

COOPERATING UNITS *(if any)*

Pediatric Oncology Branch, Medicine Branch, Clinical Pharmacology Branch, Radiation Oncology Branch

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Biology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Analysis of the histopathology of NSCLC subtypes at two Maryland institutions and in Heidelberg, West Germany, indicate that adenocarcinoma is the predominant subtype occurring currently. We have noted an apparent increase in adenocarcinomas arising in the peripheral airways (bronchioloalveolar carcinomas, BAC), and immunostaining for the major surfactant associated protein (SAP 35) confirms these findings.

B. We have established and characterized 8 cell lines from human BAC. Ultrastructural, biochemical and molecular genetic studies confirm that these cell lines are derived from the progenitor cells of the peripheral airways. These lines provide unique reagents to study the biology of these tumors and explore new methods to treat them. In addition, they may prove to be a useful commercial source for human surfactant used to treat infants with the respiratory distress syndrome.

C. A randomized prospective clinical study of extensive disease SCLC demonstrates that in vitro chemosensitivity testing accurately predicts the clinical response of the patients to initial as well as to secondary therapy. In addition, there may be a modest benefit to administering individualized drug assay based therapy. Our studies indicate that patients predicted to fail conventional initial or individualized secondary therapy may be offered alternative forms of therapy.

D. Panels of cell lines of SCLC, NSCLC and colorectal carcinomas are useful reagents to screen putative new phase I and II drugs using the MTT tetrazolium dye assay.

E. A detailed study of the mdr 1 gene in tumors and cell lines from untreated and previously treated SCLC and NSCLC patients indicates that expression of this gene is not correlated with clinical or in vitro chemoresistance in lung cancer.

PROJECT DESCRIPTION

Biology and In Vitro Growth and Drug Sensitivity Testing of Lung Cancer

Professional Staff:

PI:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
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	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
	Barnett Kramer, MD	Senior Investigator	NCI-NMOB
	John Minna, MD	Chief	NCI-NMOB

Collaborating Branches:

Pediatric Oncology Branch, Medicine Branch, Clinical Pharmacology Branch, Radiation Oncology Branch

Objectives:

One of the major objectives of this Branch is to develop newer, more rational therapies for the cancer types representing our major clinical interests. By studying the biology of specific cancers in depth, new ideas for cancer control are generated, tested *in vitro* and brought to clinical trial. We presume that such an approach is more likely to advance in the therapy of refractory tumors such as colon and non-small cell lung cancer (NSCLC) than the development of new nontargeted cytotoxic agents. Further, a comprehensive knowledge of the biology of a cancer type can aid the physician in interpreting certain clinical phenomena such as hormone secretion, tumor progression, etc. Finally, identification of tumor markers may aid diagnosis, staging, detection of relapse, imaging, subtyping, and monitoring response to therapy.

Three of the currently active clinical protocols for the therapy of SCLC and NSCLC depend on the selection of individualized patients' chemotherapy by *in vitro* drug sensitivity testing. Thus one of the major objectives of the Branch is to develop methods to 1) amplify tumor cells so that adequate numbers are available for testing; 2) develop and apply rapid, accurate, reproducible testing procedures; 3) demonstrate the clinical relevance of the testing procedures; and 4) utilize *in vitro* testing for biological and preclinical studies.

Major Findings:

The Changing Histopathology of Lung Cancer

Last year we noted an increased incidence of adenocarcinoma in our non-SCLC (NSCLC) protocol patients (63% of all NSCLC). We also noted that peripheral adenocarcinomas with some or all of the features of bronchioloalveolar (BAC) carcinomas appeared to be common (about 50% of all adenocarcinomas, or about 30% of all NSCLC tumors). We have extended these findings by reviewing the pathology of resected lung cancer at Johns Hopkins Hospital, Baltimore and at the Rohrbach Clinic, Heidelberg. The incidences of adenocarcinomas and its BAC subtype at these institutions are not significantly different from those noted at our institution.

Diagnosis and Culture of BAC Tumors

The histopathologic diagnosis of BAC tumors is difficult because these tumors can only be recognized with confidence when they are in a non- invasive or non-metastatic mode. Thus the true incidence is undoubtly far higher than stated in most reports. To confirm our findings, we have stained tumors for the major surfactant associated protein, SAP-35 (see report by Linnoila). These findings support our hypothesis that many if not most adenocarcinomas of the lung arise from the peripheral airways.

Cell culture studies support these findings. Of 16 pulmonary adenocarcinomas initiated in our laboratory that have been characterized, 8 express ultrastructural evidence of arising from or being related to the progenitor cells of the peripheral airways, either Clara cells, Type II pneumocytes, or both. These lines express SAP-35 and in some cases, the other surfactant associated proteins SP B and SP C.

In Vitro Drug Sensitivity Testing Clinical Correlation

The Weisenthal dye exclusion assay is used to test clinical specimens from patients entered onto therapeutic trials for lung cancer. The largest and best studied base currently available is from the Extensive Stage small cell lung cancer protocol, #83 13 (also see report by Dr. D. Ihde). Samples were obtained from 68/69 patients entered onto protocol (99%), and at least one tumor containing sample was obtained from 52 patients (75%). Cell lines were established from 26/52 (50%) of patients from whom at least one tumor containing specimen was obtained (38%) of all patients, and 24 of these lines (92%) had in vitro drug sensitivity testing performed. However, the in vitro best regimen ´IVBR´ was administered to 16 of these patients (67%), or 23% of all patients entered onto protocol. This protocol is highly labor intensive. Thus, while we have documented our efficiency in tumor collection, processing and culture, only 23% of all patients entered onto protocol received assay directed therapy.

DST is highly accurate in predicting patients response to initial therapy. Of 19 evaluable patients receiving initial chemotherapy (VP 16/cisplatin), 13 had partial or complete responses, while 6 were non responders. The fraction of active drugs (of 7 tested) in the responders was 68%, compared to 27% in the non-responders. Similarly, the mean percent cell kill of the tested drugs was 28% in the responders compared to 51% in the non responders.

Of patients receiving IVBR, 4 underwent a complete response (25%) compared to 2/36 (6%) receiving an empiric 3 drug combination (VAC) because DST data were not available. In part because of the small numbers, these differences are not significant, but they suggest that assay directed therapy may be more beneficial than empiric therapy.

However, DST of the patients initial specimens demonstrated that the data predicted response to the assay directed secondary therapy as well to the initial therapy. Only patients having a mean number of ´sensitive´ drugs greater than

50% and whose mean cell survival of the IVBR selected 3 drug combination was less than 50% responded to the IVBR.

DST Studies - Non Clinical

As previously described, the MTT tetrazolium dye assay has been used extensively to study the in vitro sensitivity patterns of lung and colorectal carcinomas. These studies validate the concept that cell lines are suitable models for preclinical studies. In studies performed under a collaborative agreement with Adria Laboratories, we have identified a morpholino anthracycline compound having selective toxicity for colorectal carcinomas. Colorectal lines are as sensitive as SCLC lines to this agent, while NSCLC lines are relatively resistant. These results indicate that the drug should be further investigated with an aim to bringing it into Phase I studies as soon as possible. We have also extensively tested another drug currently considered for phase I studies in lung cancer, namely ipomeanol. Ipomeanol selectively kills Clara cells present in the bronchioles of experimental animals, leading to a respiratory death. These findings suggest that ipomeanol may be of benefit in BAC tumors, especially those derived from Clara cells. We have tested several NSCLC and SCLC lines, including those derived from BAC tumors of various subtypes. Ipomeanol is relatively inactive in the MTT assay, being toxic only at concentrations near its maximum solubility. There was no selective killing of any type of lung cancer. Our prediction from these studies would be that ipomeanol is unlikely to be of great therapeutic use. We have also identified a subgroup of NSCLC (12% of tumors and cell lines) that express multiple neuroendocrine markers (NSCLC-NE tumors). These tumors are unusually sensitive in vitro to a wide range of chemotherapeutic agents, suggesting a relationship between NE properties and chemosensitivity. In collaboration with Jim Mitchell (ROB) we have found these chemosensitive tumors to be as radioresistant as other NSCLC lines. Thus these lines have the unusual phenotype of being chemosensitive but relatively radioresistant. They lack the deletions of 3p and 13q that are characteristic of SCLC tumors and carcinoids. Thus the NSCLC-NE tumors form a highly interesting subgroup, expressing properties intermediate between those of SCLC and NSCLC.

The Role of the mdr Gene in Lung Cancer

Multi-drug resistance plays a major role in the response of lung cancers to chemotherapy. While most SCLC tumors are relatively chemosensitive initially, about 20 25% exhibit de novo resistance. In addition, most previously treated SCLC tumors and most untreated NSCLC tumors are resistant.

While multiple mechanisms of drug resistance exist, one of the best studied and understood is overexpression of the mdr1 gene product. We undertook a comprehensive study of the role of mdr1 in lung cancer. We measured mdr1 mRNA levels in a large comprehensive collection of lung cancer cell lines. Complete in vitro chemosensitivity profiles and clinical patient data were available for most of the lines. A modest number of human lung cancers and corresponding normal lung tissues also were studied. Results from lung cancers were compared to colorectal lines and tumors. The vast majority of normal and tumor tissues and cell lines expressed relatively low or background levels of mdr1 mRNA. There were no significant differences noted between untreated SCLC, previously treated SCLC and NSCLC. When cell lines and corresponding tumor tissue were available for testing, data from both were similar. In contrast, colorectal lines expressed relatively high levels of mRNA. Levels in gastric lines were lower than in colorectal lines, reflecting the lower expression in normal gastric mucosa and the high levels in intestinal mucosa. Only two groups of lung lines expressed relatively high levels: two of five bronchial carcinoids and 4/5 NSCLC-NE lines. Why the NSCLC-NE lines (and 1/2 tumors) express high levels of message (as high as colorectal lines) is not understood, especially as they are as chemosensitive as untreated SCLC lines and much more sensitive than other NSCLC lines. Lung lines expressing mdrl were examined for gene amplification and rearrangements, but none were found. In summary, the mdrl gene plays little or no role in lung cancer, and the major mechanism of drug resistance must be due to other factor(s).

Future Studies

We are performing an analysis of the typing of NSCLC world wide. Sites to be analyzed will include co-operating institutions in North America, Europe and Japan. These studies will be complemented by more limited correlations between histopathology and ultrastructure and immunohistochemistry. By these techniques we will be able to confirm our light microscopic observations regarding the increase in adenocarcinomas, and its BAC subtype. Finally, we will determine whether the observations we have made are due to differences in incidence or altered diagnostic techniques, or to both. We will study the distribution of NSCLC types at Johns Hopkins Hospital in the sixties compared to recent past years.

We will continue our current clinical protocols for SCLC and NSCLC based on in vitro selected therapy. Data will be correlated with the patients' responses. Preclinical studies will include testing of phase I and II drugs and correlating in vitro predictions with clinical results. As amplification and overexpression of the myc gene family are relatively common in lung cancer, especially previously treated SCLC, we will study the relationship between oncogene expression and in vitro chemosensitivity and radioresistance. We will test both lung cancer cell lines as well as rat cell lines transfected with various oncogenes.

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1. Whang-Peng J, Freter CE, Knutsen T, Nanfro JJ, Gazdar A. Translocation t(11;22) in esthesioneuroblastoma. Cancer Cenet Cytogenet 1987;29:155-157.

2. Gazdar AF. Tumor progression and resistance to drug therapy. In: Minna J, Kuehl WM, ed. Cellular and Molecular Biology of Tumors and Potential Clinical Applications. New York: Alan Liss, 1987;297-299.

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7. Ihde DC, Russell EK, Oie HK, Linnoila RI, Steinberg SM, Ghosh BC, Schmacher HR, Minna JD, Gazdar AF. Prospective clinical trial of individualized chemotherapy based on *in vitro* drug sensitivity testing in extensive stage small cell lung cancer. In: Salmon S. ed. Adjuvant Therapy of Cancer V. Orlando: Grune & Stratton, 1987;201-207.

8. Park JG, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnson BE, Gazdar AF. Characteristics of cell lines established from human colorectal carcinomas. Cancer Res 1987;47:6710-6718.

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10. Johnson BE, Sakaguchi AY, Gazdar AF, Minna JD, Burch D, Marshall A, Naylor SL. Restriction fragment length polymorphism studies show consistent loss of chromosome 3p alleles in small cell lung cancer. J Clin Invest, in press.

11. Ernst TJ, Gazdar A, Ritz J, Shipp MA. Identification of a second transforming gene, *ras*^{n} in a human multiple myeloma line with a rearranged c-*myc* allele. Blood, in press.

12. Deftos LJ, Linnoila RI, Burton DW, Leong SS, O´Connor DT, Murray SS, Gazdar AF. Demonstration of chromogranin A in human neuroendoricne cell lines by immunohistology and immunoassay. Cancer, in press.

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15. Sausville EA, Eddy JL, Makuch RW, Fischmann AB, Schechter GP, Matthews M, Glatstein E, Ihde DC, Kaye F, Veach S, Phelps R, O´Connor T, Trepel J, Cotelingam JD, Gazdar AF, Minna JD, Bunn PA. Histopathologic staging at initial diagnosis of mycosis fungoides and Sezary syndrome: Definition of three distinctive prognosis groups. Annals Int Med, in press.

16. Linnoila RI, Mulshine JL, Steinberg SM, Funa K, Matthews MJ, Cotelingam JD, Gazdar AF. Neuroendocrine differentiation in endocrine and non-endocrine lung carcinomas. Am J Clin Pathol, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06591-03 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Second Messenger and Receptor Systems in Human SCLC

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward Sausville, MD, PhD	Senior Investigator	NCI-NMOB
Others:	John Minna, MD	Chief	NCI-NMOB
	James Battey, MD, PhD	Senior Investigator	NCI-NMOB
	Jane Trepel	Biologist	NCI-NMOB
	James Moyer, PhD	Senior Investigator	LBC-NCI
	J. Viallet, MD	Medical Staff Fellow	NCI-NMOB
	Y. Sharoni, PhD	EORTC-Fellow	NCI-NMOB

COOPERATING UNITS *(if any)*

Laboratory of Biological Chemistry, NCI; Laboratory of Pathology, NCI

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

INSTITUTE AND LOCATION
Molecular Genetics

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies with small cell lung cancer (SCLC) cell lines have demonstrated that gastrin-releasing peptide (GRP), the mammalian counterpart to bombesin, is expressed in a significant fraction of SCLC lines. To assess its role in causing autocrine stimulation, initial experiments attempted to demonstrate specific binding to SCLC membrane fractions, with little success. Accordingly, efforts to define rapid physiologic responses to GRP were undertaken. These studies demonstrated clearly that in 5/11 SCLC cell lines tested there was evidence of calcium mobilization following GRP or bombesin stimulation. The structure-activity relationship of this effect was in accord with that expected for GRP receptors in gut, brain and anterior pituitary cells. Further studies focused on the relationship of this response to inositol phosphate metabolism. In a SCLC cell line with a brisk response to GRP with increased intracellular calcium, there was evidence of increased inositol 1,4,5 trisphosphate within seconds of addition of bombesin congeners. Both the mobilization of calcium and inositol phosphate turnover were inhibited by cholera toxin and active phorbol esters. In the presence of pertussis toxin there was also a less complete stimulation of inositol phosphate turnover. In corollary studies it was demonstrated that the cell lines with the best response to bombesin showed constitutive expression of L-myc and prepro GRP without expression of c- or N-myc. In contrast, cell lines without evidence of response to bombesin had constitutive N- or c-myc expression.

These studies suggest that an order of progressively malignant and distinct phenotypes can be defined in SCLC cell lines. A most "differentiated" cell line would be those which produce and respond to GRP, and also produce L-myc. A less differentiated cell line would neither produce nor respond to GRP and express abundant c- or N-myc. GRP (+), L-myc (+), c-myc (-), and N-myc (-) cell lines would represent those in which an effort to block a potential autocrine loop involving GRP might be most successful.

PROJECT DESCRIPTION

Second Messenger and Receptor Systems in Human SCLC

Professional Staff:

PI:	Edward Sausville, MD, PhD	Senior Investigator	NCI-NMOB
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	James Moyer, PhD	Senior Investigator	LBC-NCI
	J. Viallet, MD	Medical Staff Fellow	NCI-NMOB
	Y. Sharoni, PhD	EORTC-Fellow	NCI-NMOB

Collaborating Branches:

Laboratory of Biological Chemistry, NCI; Laboratory of Pathology, NCI

Objectives:

1. To examine the mechanism of bombesin induced calcium fluxes in responding small cell lung cancer cell lines, and discern the differences in calcium storage and release between bombesin-responsive and bombesin non-responsive cell lines.

2. To define mechanisms of bombesin-induced phosphatidylinositol turnover in responding lung cancer cell lines.

3. To understand the differences between bombesin-responding and bombesin non-responding small cell lung cancer cell lines with respect to inositol lipid and inositol phosphate metabolism.

4. To examine the participation of guanine-nucleotide binding proteins in bombesin-induced signal transduction in lung cancer.

5. To characterize the bombesin-associated tyrosine kinase in small cell lung cancer cell lines, and place this in the context of other tyrosine kinase genes known which may be expressed in small cell lung cancer cells.

6. To determine if bombesin action results in altered protein kinase C activity or distribution in small cell lung cancer cell lines.

7. To determine if bombesin responsive and bombesin non-responsive small cell lines have constitutively altered protein kinase C activation, distribution, or substrates.

Methods Employed:

Calcium metabolism: Quin-2 spectrofluorometry.

Inositol Turnover: Metabolic labelling with radioisotype; high performance liquid chromatography; thin-layer chromatography.

Tyrosine kinases: Western blotting; anti phosphotyrosine antibodies; oligonucleotide synthesis and hybridization.

Guanine nucleotide binding protein: ADP-ribosylation of membrane fractions; Northern blotting with DNA-RNA hybridization; cDNA library construction.

Protein kinase C: Assays of kinase.

Major Findings:

Initial experiments have sought to define the type of calcium mobilization observed in response to bombesin in small cell lung cancer, and to correlate this with the biologic features of the available cell lines.

We have observed that bombesin congeners mobilize Ca^{2+} from intracellular stores as assayed by Quin2 in some (5/11 tested) small cell lung cancer cell lines. Not all of the Ca^{2+} appears to arise from intracellular sources, as EGTA in the external medium attenuates but does not eliminate the release of calcium. The calcium resonse, once elicited, causes the cells to be refractory to further stimulation by bombesin unless the ligand is removed. Of interest, the cell lines with the best Ca^{2+} response to bombesin have evidence of very low constitutive c- or N-myc expression, and all responders thus far assayed express abundant GRP and easily detectable L-myc transcripts. Cell lines without evidence of a Ca^{2+}-transient response to bombesin do not show GRP transcription, and have abundant c- or N-myc expression with variably occurring L-myc transcripts. Thus, these data would suggest that constitutive expression of c- or N-*myc* removes a potential need for bombesin related Ca^{2+} mobilization in promoting cell growth. It should be underscored that a major unanswered question is whether the c- or N-*myc* expression causes independence from bombesin induced stimulation or whether constitutive c- or N-*myc* expression reflects activation of a process usually under hormonal influence in those cells without constitutive c- or N-*myc* expression. The importance of this question lies in the possible pharmacologic manipulations of a system whose deregulation leads to hormone independence with c- or N-*myc* expression.

We have observed that bombesin-mediated phosphatidyl inositol (PI) turnover proceeds with initial phosphatidyl inositol bis-phosphate hydrolysis with half maximal effect at approximately 10-100 nM of added peptide. This process has been modulated in the following ways.

Active phorbol esters attenuate after short-term (15-20 min) pretreatment the bombesin mediated responses. Down-modulation of protein kinase C by protracted treatment with phorbol esters (48 hrs) eliminates this effect. This suggests that protein kinase C-responsive elements regulate bombesin induced signals in these signals. Analogous effects are observed on Ca^{2+} flux. Cholera toxin (CT) pretreated small cell lung cancer cells display marked attenuation of the bombesin-mediated Ca^{2+} flux and PI turnover. The time course of the appearance of this effect is concordant with the modification of known CT sensitive targets, such as the stimulatory guanine-nucleotide binding protein Gs.

Pertussis toxin treated small cell lung cancer cells show, at best, minimal nhibition of bombesin induced signals. Taken together, these data argue for a

CT- as opposed to PT-sensitive process in the generation of bombesin-induced signals. This would reflect participation of a toxin-sensitive G-protein, or effects of CT on lipid precursor synthesis.

We have extended our prior observation of bombesin-induced tyrosine phosphorylation in these cells to include a larger series of cell lines. The development of an increased tyrosine kinase activity correlates with effects on cell growth of bombesin and related peptides in that even in cell lines with low endogenous production of gastrin-releasing peptide, response to bombesin with increased tyrosine kinase in cell extracts occurs in cells which also respond to bombesin by increased growth.

Recent experiments have documented modulation of the bombesin response by a number of calcium channel-active compounds, including verapamil and diltiazem, and the latter compound, in particular, inhibits growth of small cell lung cancer cells with a parallel dose response relationship. These results raise the possibility of the participation of a calcium channel in the activity of bombesin to stimulate growth of small cell lung cancer.

Proposed Course:

The locus of activities to be shifted to Georgetown University where extra-mural funding mechanisms will be sought.

Publications:

1. Heikkila R, Trepel JB, Cuttitta F, Neckers LM, Sausville EA. Bombesin-related peptides induce calcium mobilization in a subset of human small cell lung cancer cell lines. J Biol Chem 1987; 262:16456-16460.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06594-03 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetic Events in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB
	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*
Jaqueline Peng, M.D., Medicine Branch, COP, DCT, NCI; Bert Zbar, M.D., Immunobiology Branch, BRMP, DCT, NCI; Susan Naylor, Ph.D., Department of Cellular and Structural Biology, Univ. of Texas

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Human Tumor Biology and Molecular Genetics and Immunology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.4	0.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Sixty-six tumor cell lines from small cell lung cancer patients have been studied for myc family DNA amplification. Fifteen of 35 (43%) cell lines established from previously treated small cell lung cancer patients have been amplified for one of the myc family genes compared to 3/30 (10%) of cell lines established from untreated patients. Chemotherapy treated small cell lung cancer patients whose tumor cell lines have c-myc family DNA amplification live a median of 27 weeks compared to 49 weeks for patients whose tumor cell lines do not have c-myc family DNA amplification. Thirty-eight tumors harvested from small cell lung cancer patients have been studied for myc family DNA amplification. Six of 38 (16%) have DNA amplification of one of the myc family of oncogenes (4 N-myc and 2 L-myc). All these were obtained from chemotherapy treated small cell lung cancer patients. myc family DNA amplification appears to be more common in chemotherapy treated small cell cancer patients and c-myc family DNA amplification is associated with shortened survival.

B. Restriction fragment length polymorphism (RFLP) studies have shown that 33 of 35 patients with small cell lung cancer lose heterozygosity in the tumor tissue DNA that was present in normal tissue DNA. This confirms the loss of genetic information localized to the short arm of chromosome 3 previously described by karyotype analysis. Extrapulmonary small cell cancers have been studied using karyotype and RFLP analysis and 4/5 patients retain both chromosome 3's in the region of 3p14-21 by both RFLP analysis and karyotype analysis. This demonstrates a different genetic lesion in tumors that histologically are indistinguishable.

PROJECT DESCRIPTION

Molecular Genetic Events in Lung Cancer

Professional Staff:

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB
	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Jaqueline Peng, M.D., Medicine Branch, COP, DCT, NCI; Bert Zbar, M.D., Immunobiology Branch, BRMP, DCT, NCI; Susan Naylor, Ph.D., Department of Cellular and Structural Biology, Univ. of Texas

Objectives:

1. Determine the myc family DNA amplification pattern in the new, prospectively characterized lines.

2. Update the information on the clinical association specifically to see if the amplification pattern changes with the new VP/PLAT induction regimen compared to the CMC-VAP regimen.

3. Simultaneously study normal tissue, tumor cell lines, and tumors from the patients for chromosomal deletions using restriction fragment length polymorphisms while having a karyotype performed on the cell line to correlate the evidence for chromosomal deletion using restriction fragment polymorphisms and cytogenetic analysis.

Methods:

We have completed analyzing the 66 tumor cell lines established from small cell lung cancer patients´ tumors treated on protocol at the NCI-Navy Oncology Branch and correlated this with the clinical presentation and course of these patients. We initially grew up all 66 cell lines and prepared DNA. The DNA was hybridized to radiolabelled fragments of the myc family of genes, c-myc, N-myc, and L-myc. DNA copy number was determined relative to a diploid copy control. DNA was considered to be amplified if the signal from the myc family gene was 4 fold greater than a single copy control.

Major Findings:

myc Family DNA Amplification is More Common in Cell Lines Established from Chemotherapy Treated than Untreated Patients and c-myc Amplification is Associated with Shortened Survival

The DNA from 18 of the 66 small cell lung cancer cell lines were amplified for one of the myc family oncogenes (7 c-myc, 5 N-myc, and 6 L-myc). myc family DNA amplification was more common in cell lines established from chemotherapy treated than untreated patients (15/35 versus 3/30 respectively). The survival of patients whose cell lines were established after treatment with chemotherapy had c-*myc* amplification was shorter than other patients whose cell lines did not (median 27 versus 49 weeks). The survival of chemotherapy treated patients whose cell lines had N-*myc* or L-*myc* amplification was not markedly different than the patients whose cell lines were not.

myc Family DNA Amplification in Tumors is the Same as in the Cell Lines Established from the Same Patients

We have collected 38 tumors from small cell lung cancer patients adequate for molecular genetic analysis, 4 from surgical biopsies and 34 from postmortem examinations. Thirty-eight have been analyzed for *myc* family DNA amplification Thirty-two tumors were obtained from patients who had been treated with chemotherapy and six were from patients who had not received chemotherapy. Six of 32 tumors from chemotherapy treated patients had DNA amplification of one of the *myc* family oncogenes, 4 N-*myc* and 2 L-*myc*. None of the 6 tumors from untreated small cell lung cancer patients had any *myc* family DNA amplification. Nine of the patients had both tumors and tumor cell lines studied. All nine had the same *myc* family DNA amplification pattern in their tumor cell line and their tumor. Two had N-*myc* DNA amplification in both the tumor and tumor cell line from the same patient and the other seven had a diploid copy of the *myc* family genes in both their tumor and tumor cell line.

Restriction Fragment Polymorphism Studies Show Consistent Deletion of Loci on the Short Arm of Chromosome 3 in Small Cell Lung Cancer Patients′ Tumors Compared to Their Normal Tissue

In collaboration with Dr. Sue Naylor and Dr. Berton Zbar, we have prepared DNA from normal and tumor tissue or tumor cell line from the same patient and analyzed the tumor DNA for loss of heterozygous loci on chromosome 3p in the tumor tissue compared to normal tissue from the same patient. They have evaluated the normal tissue DNA with DNA fragments that detect heterozygous loci on chromosome 3p, D3S2, D3S3, and DNF15S2. The normal tissue had heterozygous loci within chromosome 3p14-23 in 35 of the 37 DNAs analyzed. The tumor was reduced to homozygosity in one of these loci in 33 of the 35 patients (94%). Thus, by using this restriction fragment polymorphism analysis, the tumor DNA shows loss genetic information from at least a portion of the short arm of one chromosome 3 in 33 of the 37 patients. This lends support to the previous karyotypic description of a consistent deletion of the short arm of chromosome 3 in small cell lung cancer.

The Short Arm of Chromosome 3 is not Lost in Extrapulmonary Small Cell Cancer

One of the patients who did not lose heterozygosity in this tumor tissue DNA compared to the normal tissue DNA was a patient who had extrapulmonary small cell cancer arising in his prostate gland. Therefore, we identified 5 extrapulmonary small cell cancer patients who had tumor cell lines or tumor plus normal tissue available for study. These 5 patients had their tumors arise in the prostate gland, cervical lymph nodes, brain, uterus, and cervix. We then studied the patients′ 4 tumor cell lines with karyotype analysis. The 4 tumor cell lines, 3 tumors and 3 normal tissue DNAs were studied with three probes which detect restriction fragment length polymorphisms within 3p14-21. The karyotype analysis showed all 4 tumor cell lines retain at least 2 chromosome 3's in the 3p14-21 region. The restriction fragment length polymorphism studies demonstrated the presence of heterozygous alleles in the 3p14-21 region with one exception. The patient who presented with small cell cancer in their cervical lymph nodes showed a reduction to homozygosity in this tumor DNA at the $D3^S2$ locus (3p14-21). We believe it is interesting that this was the only patient who presented with metastases to the lymph nodes and did not present with an identifiable primary small cell cancer like the other 4 patients studied.

These studies of extrapulmonary small cell cancer have shown that in contrast to the consistent deletion of the 3p14-23 region in small cell lung cancer, extrapulmonary small cell cancer typically retains both arms of chromosome 3.

Publications:

1. Johnson BE, Ihde DC, Makuch RW, Carney DN, Oie H, Russell E, Nau MM, Minna JD. myc family oncogene amplification in tumor cell lines established from small cell lung cancer patients and its relationship to clinical status and course. J Clin Invest 1987;79:1629-1634.

2. Naylor SL, Johnson BE, Minna JD, Sakaguchi AY. Loss of heterozygosity of chromosome 3p markers in small cell lung cancer. Nature 1987;329:451-454.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06595-02 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinically Relevant Immunohistochemical Markers in Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Human Tumor Biology

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our goal is to define immunohistochemical markers that will best type lung cancer for diagnosis, prognosis, and selection of therapy. Small cell lung cancer (SCLC), characterized by neuroendocrine (NE) features, is responsive to chemo- and radiotherapy. Some non-SCLC also express NE features. The hypothesis is that these tumors might be more responsive to cytotoxic treatment than other non-SCLC.

A. Characterization of markers. In a retrospective study a comprehensive group of 113 lung cancers were tested for the immunohistochemical expression of 17 antigens using a sensitive avidin-biotin-peroxidase technique. Logistic regression analysis was used to separate tumors into the proper categories (SCLC and carcinoid tumors versus NSCLC) based on the immunohistochemical markers. As a result 95% of the tumors were correctly predicted using the cell counts and staining intensities of only six markers. The results suggested that 1) individual marker counts are not useful in tumor classification, 2) "specific" NE markers such as serotonin and neuropeptides bombesin, calcitonin, ACTH, vasopressin, neurotensin are not useful, 3) the best NE markers are a panel of "general" NE markers (Chromogranin A, Leu 7, NSE) which are present in NE cells throughout the body.

B. Clinicopathologic correlation. This panel of "general" NE markers were applied to the non-SCLC cases on protocol 83-15 in our branch. There was a concordant expression of immunohistochemical NE markers with other biochemical tests used to characterized NE differentiation such as L-dopa decarboxylase activity. Although the numbers were small, the response rate to chemotherapy was 60% (3/5) in the patients whose tumors were positive for NE markers versus 20% (4/21) in those with negative NE markers.

The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. Immunohistochemistry provides a highly effective and specific technique to achieve this goal.

PROJECT DESCRIPTION

Clinically Relevant Immunohistochemical Markers in Lung Cancer

Professional Staff:

PI:	Ilona Linnoila, MD	Senior Investigator	NCI-NMOB
Others:	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Adi Gazdar, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Biostatistics and Data Management Section, Clinical Oncology Program, DCT, NCI, (Seth Steinberg, PhD), Anatomic Pathology, Naval Hospital and Anatomic Pathology, NCI, NIH

Objectives:

There are four major histological types of lung cancer, namely small cell lung cancer (SCLC) (25%), adenocarcinoma (25%), squamous cell carcinoma (30%) and large cell carcinoma (15%). For a number of biological and clinical reasons, these lung carcinomas may be divided into SCLC and non-SCLC (NSCLC) tumors. SCLC and the rare bronchial carcinoid express many neuroendocrine (NE) features including dense core granules by electron microscopy, high levels of the key amine producing enzyme L-dopa decarboxylase, and the glycolytic isoezyme neuron-specific enolase (NSE) and hormone or neuropeptide production.

SCLC unlike NSCLC is extremely sensitive for chemotherapy and radiation, and there are scattered reports on favorable responses to chemotherapy by "atypical endocrine" tumors of the lung. This knowledge together with the recently established NE markers has prompted us to explore if 1) the expression of neuroendocrine markers in NSCLC is associated with favorable response to chemo- or radiotherapy, and 2) if the degree of expression of neuroendocrine markers in SCLC correlates with clinical outcome. Immunohistochemical technique provides a readily applicable tool for this.

Methods Employed:

1. Tumors. A comprehensive group of 113 primary lung cancers was chosen from the archives of the departments of pathology at the Bethesda Naval Hospital and National Cancer Institute. In addition, tumor material was obtained also from the patients on NCI protocol 83-15. Serial sections from routinely processed paraffin blocks were used.

2. Antibodies. The application of immunologic techniques that use hormone markers has been hampered by the fact that tumors with similar histologic and cytologic features may produce a variety of immunoreative substances, and some tumors may synthesize more than one hormone. Recently, a mouse monoclonal antibody LK2K10 produced against human pheochromocytoma has been shown to be directed against chromogranin (ChrA) a constituent of secretory granules in most peptide producing endocrine cells. The demonstration of chromogranin in lung tumors serves as a useful marker for a broad spectrum of lung tumors with NE

features including SCLC and the rare bronchial carcinoid. Other general immunohistochemical markers for NE differentiation include monoclonal antibody to Leu-7 (HNK-1). Leu-7 was originally identified in subpopulation of lymphocytes called natural killer cells and later noted to be present also in nerves and wide variety of endocrine cells. Antibodies to NSE also react with nerves and cells of the diffuse NE system and its tumors. The advantage of applying such general NE markers in that they provide a more uniform recognition for multiple NE tumors that may in turn synthesize a variety of specific products such as different hormones.

3. Immunohistochemical Staining. Staining was performed using the avidin-biotin peroxidase technique. Appropriate positive and negative controls were included in each assay. Results of the immunostaining were reviewed scoring both for the intensity of the staining and number of positive cells.

Major Findings:

1. Characterization of Markers. We were able to demonstrate that the majoritiy of cells in most SCLC and all carcinoid tumors were positive for the general NE markers and many hormones. Logistic regression analysis was used to separate tumors into the proper categories on the basis of markers and 95% the tumors were correctly classified applying model created from staining indeces of general NE markers (ChrA, Leu 7, NSE). Evaluation of the expression of multiple markers revealed that 7/77 NSCLC had a staining pattern indistiguishable from SCLC.

We have concluded that 1) Application of the general NE markers produces acceptable classification of lung tumors; 2) Most but not all SCLC and carcinoids express multiple NE markers in a high percentage of tumor cells; 3) Occasional NSCLC show staining patterns indistinguishable from SCLC; 4) Many NSCLC contain a small subpopulation of cells expressing NE markers.

2. Clinicopathologic Correlation. The panel of "general" NE markers (ChrA, Leu7, NSE) was applied to the non-SCLC cases on protocol 83-15 ("Treatment of Non-Small Cell lung Cancer Utilizing In Vitro Drug Sensitivity"). Based on a detailed histopathological evaluation of tumor specimens of the patients already entered in the protocol it appears than in over 80% of the cases such an immunohistochemical analysis on untreated patient specimens can be performed. Currently we have stained 101 of the 133 cases entered and in 20/98 (20%) non-SCLC at least two out of the three general NE markers were positive. The results of the first 80 cases are summarized in the following table as an example:

GENERAL NE MARKERS IN NSCLC BY HISTOLOGICAL TYPE
(80 CASES ON PROTOCOL 83-15)

(% positive)	Chr A	Leu 7	NSE
Adenocarcinoma	2/45 (4)	10/45 (22)	22/45 (49)
Large cell	6/19 (32)	3/19 (16)	9/19 (47)
Epidermoid	0/11 (0)	2/11 (18)	4/11 (36)
Other	0/2 (0)	0/2 (0)	1/2 (50)
TOTAL NSCLC	8/77 (10)	15/77 (19)	35/77 (45)
Carcinoid	3/3 (100)	3/3 (100)	3/3 (100)

The updated analysis of the response rate to chemotherapy in those 122 non-SCLC patients on protocol 83-15 in correlation with the results of immunohistochemistry revealed a rate 50% (4/8) in the patients whose tumors were positive for at least 2 out of 3 general NE markers versus 21% (7/34) in those with negative NE markers. There was also a strong correlation of the expression of immunohistochemical NE markers with other biochemical markers for NE differentiation, such as L-dopa decarboxylase levels in tumors.

Significance to Biomedical Research and the Program of the Institute:

The significance of the project lies in the possible identification of prognostically important clinical subsets of lung cancer. There are at least 150,000 new cases of lung cancer (75% of which are non-SCLC) discovered annually. Our preliminary results support our hypothesis that non-SCLC which express NE features might be more responsive to cytotoxic treatment than other non-SCLC. Immunohistochemistry provides a highly effective and specific manner to screen for these tumors.

Proposed Course:

1. The expression of markers will be correlated to the clinical data including performance status, best response, and survival. At the end of the protocol 83-15 we should have accumulated results on 120 patients, if 150 patients are accrued as planned. This will provide a basic correlation.

2. Based on our initial observations we expect that 10-20% of non-small cell lung cancers express neuroendocrine markers. In order to extend the analysis and reach meaningful clinical correlations we have initiated a collaboration with the ECOG (Eastern Cooperative Oncology Group) and LCSG (Lung Cancer Study Group). ECOG and LCSG have full clinical response and survival information on nearly 2.800 treated patients. A large number of these have pretreatment tumor samples available for analysis. We plan to study the expression of general NE markers chromogranin, Leu 7 and NSE and related this to tumor type, response to therapy, and survival. At the present time, we have stained nearly 400 cases, and the interim analysis revealed that 12-16% of the cases were positive for at least two out of the three general NE markers, thus confirming our initial findings in independent tumor sets.

Publications:

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2. Mulshine JL, Keenan AM, Carrasaquilla JA, Walsh T, Linnoila RI, Holton OD, Harwell J, Larson S, Bunn PA, Weinstein JN. Immunolymphoscintigraphy of pulmonary and mediastinal lymphnodes: A new approach to lung cancer imaging. Cancer Res 1988;47:3572-3576.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06596-02 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)
In Vitro Drug Testing for Limited SCLC and Phase I Drug Development

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB

COOPERATING UNITS (*if any*)
Eli Glatstein, M.D., Jane Grayson, M.D., and Karen Strauss, M.D., Radiation Oncology Branch. John Strong, Ph.D., Robert Parker, Ph.D., Biological Chemistry Branch and Hoo Geung Chun, M.D., Investigational Drug Branch

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Human Tumor Cell Biology and Molecular Genetics and Immunology

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS	PROFESSIONAL.	OTHER
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

A protocol combining twice a day radiotherapy plus VP 16 and cisplatin for limited stage small cell lung cancer continues. Eighteen patients have been entered onto study and 13 of 14 patients who have completed therapy have achieved a complete remission. 14 of 18 patients are alive and free of cancer or undergoing induction therapy with a median follow up of 10 months. No patients have been hospitalized for combined modality pneumonitis.

A phase I trial using dihdrolenperone, an agent identified as being active against human lung cancer by the human tumor colony forming assay (HTCFA) has been initiated. Twenty-three patients have been studied at 6 dosage levels. The principle side effects have been somnolence and hypotension in all patients. Two patients have had to stop therapy because of somnolence and none because of hypotension. Preliminary pharmacokinetic determinations show peak absorption at 3 hours and that level vary less than 50% over the 12 hours dosing interval.

From this studies we conclude that the HTCFA has identified a compound with novel side effects and the maximum tolerated dose has not been reached.

PROJECT DESCRIPTION

In Vitro Drug Testing for Limited SCLC and Phase I Drug Development

Professional Staff:

PI:	Bruce E. Johnson, MD	Senior Investigator	NCI-NMOB
Others:	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	Adi F. Gazdar, MD	Senior Investigator	NCI-NMOB
	John D. Minna, MD	Chief	NCI-NMOB

Collaborating Branches:

Eli Glatstein, M.D., Jane Grayson, M.D., and Karen Strauss, M.D., Radiation Oncology Branch. John Strong, Ph.D., Robert Parker, Ph.D., Biological Chemistry Branch and Hoo Geung Chun, M.D., Investigational Drug Branch

Objectives:

1. Determine the frequency with which adequate tumor tissue can safely be obtained and drug sensitivity data determined.

2. Determine the response rate, toxicity, and survival of limited stage small cell lung cancer patients treated with VP/PLAT, simultaneous twice a day chest radiotherapy, and chemotherapy based on intro drug testing or a standard regimen (VAC).

3. Determine changes in drug sensitivity when tumor is obtained pre and post-treatment from the same patient.

4. Determine the side effects and maximum tolerated dose of dihydrolenperone.

5. Determine the pharmacokinetics of orally administered dihydrolenperone.

6. Determine the activity of dihydrolenperone within the confines of a Phase I trial.

7. Determine the correlation between the in vitro determined activity of dihydrolenperone and the patient response.

Methods:

A. Study Design

1. Small cell lung cancer patients undergo staging.
2. Limited stage patients undergo surgical biopsy of tumor tissue.
3. Induction with 12 weeks of VP-16/PLAT with concomitant 150 RAD twice a day radiotherapy to 4500 RAD over 19 days.
4. Patients with in vitro drug sensitivity data receive an additional 12 weeks of the in vitro best regimen, patients with no in vitro data receive 12 weeks of a standard vincristine, doxorubicin, and cyclophosphamide regimen.

5. Patients are followed for survival and toxicity.
6. Small cell lung cancer patients failing conventional combination chemotherapy and non-small cell lung cancer patients for whom no curative therapies are available are identified for Phase I drug trial.
7. Patients are treated orally twice a day for 28 days with dihydrolenperone and observed for toxicity.
8. Patients with tumor tissue available have in vitro testing with DHLP performed.

Major Findings:

The Limited Stage Small Cell Lung Cancer Trial Administering VP 16 and Cisplatin Plus B.I.D. Chest Radiotherapy Has a High Response Rate and Has Decreased the Incidence of Potentially Fatal Pulmonary Toxicity

The limited stage study was approved to enter patients in July of 1986. Eighteen patients have been entered to date and 14 have completed their course of chest radiotherapy and 24 weeks of chemotherapy. Thirteen of 14 patients who have completed 24 weeks of their therapy have had a complete response. The patient who did not achieve a complete response has died of progressive small cell lung cancer. One patient who achieved a complete response relapsed systemically and died of progressive tumor. In addition, two patients have relapsed in the CNS only without evidence of a systemic relapse. The other patients are alive and free of cancer or undergoing their initial treatment with a median follow up of 10 months (range 2-22 months). The toxicity compared to the previous combined modality trial in small cell lung cancer has been dramatically reduced. Two of the 18 patients have developed pulmonary toxicity requiring steroid treatment but have not needed hospitalization and only two patients have been hospitalized for neutropenic fevers.

Phase I Trial of Dihydrolenpoerone, A Novel Compound Active Against Lung Cancer Identified by the Human Tumor Colony Forming Assay

The phase I and pharmacokinetic study of dihydrolenperone was approved to enter patients in January of 1986. Twenty three lung cancer patients have been entered to date and have completed 22 courses of dihydrolenperone. The initial dose was 10mg/m^2 orally twice a day for 28 days. The dosage has been escalated to 20mg/m^2, 30mg/m^2, 40mg/m^2, 50mg/m^2, and currently 60mg/m^2 b.i.d. The prominent side effects have been somnolence and hypotension in all patients. The hypotension observed in the first two patients treated with 20mg/m^2 was 70/50 when the patients were standing. This prompted us to alter the loading schedule with dihydrolenperone so the dosage was increased 10mg/m^2 twice daily until the target dosage is reached. The hypotension with this schedule has been more tolerable. Somnolence has been noticeable in all patients with more pronounced somnolence in patients taking narcotics. Two patients discontinued the drug because of somnolence. One patient who was taking methadone for pain caused by tumor involving the liver discontinued taking dihydrolenperone because of somnolence causing him to sleep more than half the day. Another patient discontinued the DHLP and her symptoms revsolved. One other additional patient developed rather severe depression while taking the drug with a flat affect and inability to intiate activities. This developed over the Christmas and New Years holidays. He finished the course of therapy and the depression continued after he stopped the drug. He is currently on Elavil therapy.

Two additional patients did not complete their prescribed courses of dihydrolenperone. One patient developed nausea and vomiting and his drug was stopped. He died within 3 weeks and his autopsy showed tumor constricting his small intestine leading to an intestinal obstruction. The other patient died suddenly two days after his hospital discharge after starting dihydrolenperone. Autopsy showed a massive pulmonary embolus as a cause of death. There has been no observable hematologic, pulmonary, renal, or hepatic toxicity at the dosage levels studied. The pharmacokinetic studies by John Strong of the Biologic Chemistry Branch using HPLC have demonstrated that peak absorption approximately 3 hours after the oral dosage. In addition the twice a day schedule gives drug levels fluctuating less than 50% during the day. The determination of a half life awaits additional studies at higher dosage levels where the drug can be measured more easily. Eighteen different patients have completed at least one course of therapy and are available for analysis of response to therapy. Nine patients had no response to therapy and nine developed progressive disease. We plan to continue to administer DHLP until the maximum tolerated dose.

Publications:

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2. Johnson BE, Minna JD. Small cell lung cancer. In: Fishman A, ed. Pulmonary Diseases and Disorders. New York: McGraw-Hill, 1988;1971-1982.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-06597-02 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Non Small Cell Lung Cancer Therapy Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Reserach Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB
	Henry Stevenson, MD	Senior Investigator	NCI-NMOB

COOPERATING UNITS *(if any)*

Anatomic Pathology, NHBETH (J. Cotilingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (E. Woods); Radiation Oncology Branch, Surgery Branch, (J. Grayson); Clinical Oncology Program

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Clinical Investigations (Section Head: Daniel Ihde)

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A primary objective of this Branch is to improve the state-of-the-art in the therapy of lung cancer. In the past, this Branch had focused this effort in the study of small cell lung cancer. With the advances both in the therapy of the small cell patients as well as in the study of small cell lung cancer biology, we decided to generalize the Branch effort to include the systemic evaluation of non-small cell lung cancer. The vehicle for this pilot study of the feasibility and value of using in vitro criteria to select therapy for patients with metastatic non-small cell lung cancer.

PROJECT DESCRIPTION

Non Small Cell Lung Cancer Therapy Project

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Other:	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Herbert Oie, PhD	Research Biologist	NCI-NMOB
	Edward Russell	Reserach Biologist	NCI-NMOB
	Mae Jean Englee	Biology Lab Technician	NCI-NMOB
	Sandra Jensen	Biology Lab Technician	NCI-NMOB
	Henry Stevenson, MD	Senior Investigator	NCI-NMOB

Collaborating Branches:

Anatomic Pathology, NHBETH (J. Cotilingham); Pulmonary Medicine, NHBETH (T. Walsh); Thoracic Surgery, NHBETH (E. Woods); Radiation Oncology Branch, Surgery Branch, (J. Grayson); Clinical Oncology Program

Objectives:

1. To improve therapy of non small cell lung cancer by selecting chemotherapy on the basis of in vitro analyses, both of drug sensitivity and neuroendocrine markers. To use that protocol as a vehicle for the in-depth study of small cell lung cancer biology.

2. Pilot study to evaluate if patients treated on the basis of their tumor cells´ in vitro response to a panel of chemotherapeutic agents have more effective tumor cytoreduction than conventionally treated control patients or historic controls.

3. To determine if non-small cell lung cancer patients with tumors expressing neuroendocrine features characteristic if small cell lung cancer experience natural history more typical of small cell lung cancer.

4. To evaluate our ability to prospectively establish clinical specimens as long-term cell lines.

   a. Optimizing our ability to grow specimens, especially in developing serum-free media systems.

   b. Use the computerized clinical and laboratory data bases to correlate

Methods Employed:

1. Clinical trial

2. In vitro drug sensitivity analysis

3. Immunohistochemistry

4. Biochemical Marker analysis

5. Cell culture

Major Findings:

Since this study opened in April, 1984, over 100 patients have been accrued to this study. We performed an interim analysis for the first 90 patients on the study.

As a function of protocol design, all patients had tumor tissue come to the laboratory. In several instances, the tumor tissue non-viable due to immersion in formaldehyde, but excellent cooperation between surgeons and pathologists resulted in a better than 95% yield. In order to obtain tissue from as many patients as possible, we frequently obtain tissue from patients undergoing potential curative thoracic resection. We treat only those patients with metastatic disease that is measurable or can be evaluated. Of the 35 patients who already received chemotherapy on this protocol, tumor tissue arrived in the lab was of sufficient size and condition to do at least limited in vitro drug sensitivity analysis in 29%. Some patients have relapsed and died without any chemotherapy (5 patients) and many more are still followed without any evidence of recurrent disease (39 patients). We have established continuous cell lines on 23% of the patients we have evaluated. We project that the frequency of successfully performing in vitro analysis with our current approach may increase to 40% of the total prospective cases. Further refinements of this approach will be necessary to permit this approach to be more generally applicable and we will outline some of the research directions we are pursuing to accomplish this.

These cell lines are a very useful recourse in conducting further experiments to improve the frequency of successful drug sensitivity analysis. First, the initial cell lines derived in the course of non-small cell protocol are being used in validation of another technique of drug sensitivity analysis, the semi-automatic colorimetric assay. This work will be discussed elsewhere in this document, but there are two areas in which the work with this assay impacts on the non-small cell clinical trial. First, this assay requires significantly less operator time to perform, has a more objective mode of analysis, and ultimately may require a smaller number of tumor cells for analysis. Due to the efficacy of this technique, we might also be able to achieve the goal of testing combinations instead of single agents in vitro. For these reasons, we are motivated to substitute this assay for the dye exclusion assay, after we determine the degree of comparability between the two assays. Second, we have used this assay to examine the growth factor requirement of small cell lung cancer to optimize a serum-free media system for those cells. We are now ready to extend this approach to non-small cell tumors as it is apparent from our low rate of successfully generating tumor cell lines that our current media systems are suboptimal. Both of these adjustments, a more efficient in vitro assay and a more effective media system, have the potential of improving the biggest shortcoming of this approach, this is, increasing the percentage of cases that we can successfully test for drug sensitivity in vitro.

As discussed previously, the number of patients actually receiving the combinations of drugs selected by the assay as being most active (based on single agent activity) is small (8 patients). This number will increase since we plan to accrue another 50 patients and as more of the patients, who underwent potentially curative thoracic resection, develop recurrent disease. Nevertheless, the results of the in vitro analysis suggested their tumors would be minimally responsive. The eight predicted most active combinations resulted in only a half log of cell kill in vitro.

70% of the single agents tested with these 8 tumors were resistant by our arbitrary scale (resulting in less than 50% tumor cell kill). None of these patients had an objective tumor response, but their median survival was five months. The survival rate was equivalent to the patients who received empiric etoposide/cisplatin. Since we are still dealing with small numbers of patients, we have not evaluated the two groups for the equivalence of prognostic features, so it is too early to conclude anything about the utility of the in vitro drug selection to see if a trend emerges. This study, which is really a pilot effort, will not definitely answer the questions regarding the clinical value of in vitro drug sensitivity analysis, but it will provide a departure point for constructing subsequent clinical trials to further resolve such issues.

One of the most provocative directions explored in this study is the prospective evaluation of the fate of the subset of non-small cell lung cancer without biochemical features of small cell cancer. We have prospectively analyzed cell tissues obtained in this study for the expression of four biochemical features generally felt to be characteristic of small cell. Based on the previous retrospective work in characterizing these biochemical markers, expected this phenomenon would be present in about 15% of clear cut non-small cell lung cancers. Our hypothesis was that the patients with these tumors would respond to their treatments in a fashion similar to small cell lung cancer patients (i.e. a higher response rate). We were able to do at least one biochemical parameter on 71 of 81 adequate tumors (88%). 11% of these specimens had elevated levels of expression of at least one biochemical marker. Seven non-small cell lung cancer parts with neuroendocrine biochemical features were treated with a combination chemotherapy used extensively in the Branch for small cell lung cancer (cytoxan, methotrexate, CCNU, vincristine, adriamycin, procarbazine). The response rate has been 43% for those seven "neuroendocrine" patients versus 11% for the remaining 28 non-small cell lung cancer patients treated to date on this study with corresponding median survival rate of 9 months versus 6 months. Considerable work has been done with the in vitro characterization of these neuroendocrine non-small cell lung cancer cell lines, especially in regard to their in vitro drug sensitivity. This will be discussed elsewhere in this document.

Significance to Biomedical Research and the Program of the Institute:

Lung cancer is the leading cause of cancer mortality in our society. Non-small cell lung cancer which comprises 75% of all lung cancer is universally fatal once it has metastasized. Despite intensive clinical reaearch, no major improvement has occurred in the treatment of disseminated non-small cell lung cancer. To address this the NCI-Navy Medical Oncology Branch has attempted to integrate a systemic effort to study the biology of this cancer in conjugation with an attempt to optimize the best available treatment. This entails testing a patient's tumor tissue in the laboratory for its response to standard chemotherapy agents. Based on the in vitro result, a combination is constructed that represents the most cytotoxic single agents for a particular patient's tumor.

This approach potentially has general merit in attempting to specifically tailor available treatments to the unique biology of a patient's tumor. This approahc also insures tumor tissue comes to our laboratory and is potentially available to be established as a continuous cell line. These cell lines are excellent model systems for a variety of laboratory investigations.

Proposed Course:

Further accrual of patients to the ongoing protocol will continue. More experience with the process of in vitro drug sensitivity testing to select patient therapy is required both to further analysis of its further refine the process as well as allow fuller analysis of its benefit.

Independent validation of the enhanced initial responsiveness to chemotherapy of patients whose tumor empresses neuroendocrine differentiation is required to corroborate the preliminary clinical trial outcome. To accomplish this, collaborations have been developed with two cooperative groups to analyze for the expression of neuroendocrine features from tumor specimens obtained from patients already treated with chemotherapy. The goal would be examine if the correlation over neuroendocrine expression with enhanced responsiveness to chemotherapy. Further associated biological studies will also proceed.

Publications:

1. Gazdar AF, Tsai C-M, Park J-G, Ihde DC, Mulshine JL, Carmichael J, Mitchell J, Minna JD. In vitro assays for predicting clinical response in human lung cancer. In: Peters L, Chapman D, eds. Prediction of Tumor Treatment Response. New York: Permagon Press, in press.

2. Linnoila RI, Mulshine JL, Steinberg SM, Funa K, Matthews MJ, Cotelingham. Neuroendocrine differentiation in endocrine and non-endocrine lung carcinomas. Am J Clin Path, in press.

3. Gazdar AF, Hellman L, Israel M, Russell E, Linnoila I, Mulshine JL, Schuller H, Park J-G. Expression of neuroendocrine cell markers L-Dopa decarboxylase chromogranin a and dense core granules in human tumors of endocrine and non-endocrine origin. Cancer Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06598-02 NMOB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Senior Scientist	NCI-NMOB
	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	Barnett Kramer, MD	Senior Investigator	NCI-NMOB
	Ed Sausville, MD, PhD	Investigator	NCI-NMOB
	Ingalill Avis, RN	Biologist	NCI-NMOB

COOPERATING UNITS (if any)
Radiation Oncology Branch, (E. Glaltstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian, D. Hoth)

LAB/BRANCH
NCI-Navy Medical Oncology Branch

SECTION
Clinical Investigations (Section Head: Daniel Ihde)

INSTITUTE AND LOCATION
NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The efforts of this Branch has been central to the recognition of gastrin releasing peptide as an autocrine growth factor for small cell lung cancer (7). Dr. Cuttitta developed a monoclonal antibody (2A11) to the active portion of that peptide and demonstrated that the immunoglobulin could block the mitogenic effect of GRP in vitro and in vivo. We have recently, in collaboration with Hybritech, Inc. (San Diego, CA), initiated a clinical trial to test whether one can control autocrine mediated malignant proliferation of small cell lung cancer using a monoclonal antibody. Our Branch has a long standing interest in the role of growth factors in cancer, so that information from 2A11 antibody clinical trial could be a foundation from subsequent anti-growth factor trials.

PROJECT DESCRIPTION

Diagnostic and Therapeutic Clinical Trials with Monoclonal Antibodies - Part I

Professional Staff:

PI:	James L. Mulshine, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Ilona Linnoila, MD	Pathologist	NCI-NMOB
	Frank Cuttitta, PhD	Senior Scientist	NCI-NMOB
	Daniel C. Ihde, MD	Senior Investigator	NCI-NMOB
	Barnett Kramer, MD	Senior Investigator	NCI-NMOB
	Ed Sausville, MD, PhD	Investigator	NCI-NMOB
	Ingalill Avis, RN	Biologist	NCI-NMOB

Collaborating Branches:

Radiation Oncology Branch, (E. Glaltstein); Nuclear Medicine, Clinical (J. Carrasquillo); FCRF (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian, D. Hoth)

Objectives:

1. To study the pharmakinetics of monoclonal antibody delivery, attempting to maximize delivery of antibody to tumor involve sites.

2. To study methods of radiolabeled monoclonal antibody imaging as a staging tool in evaluating patients with cancer.

3. To determine if monoclonal antibody can be used to block growth factor stimulated tumor proliferation.

4. To study tumor cells to identify other growth factors which are potential targets for immunomolecular regulation.

Methods Employed:

1. Radionuclide Imaging

2. Immunohistochemistry

3. Radioimmunoassay

4. Radioautography

Major Findings:

A. This Branch was involved in an early monoclonal antibody therapy trials in cutaneous T-cell lymphoma to determine if the monoclonal antibody could mediate cytoreduction by enhancing immune clearance of malignant T-cell. This trial failed to demonstrate significant therapeutic benefit, but did provide a vehicle for successful diagnostic imaging studies. The initial diagnostic imaging studies were developed by Paul Bunn, M.D., and continued by Dr. Mulshine. The intravenous delivery of In^{111} labeled T101 has resulted in the highest percent of tumor targeting achieved as of the time of its reporting. This localization efficiency was further therapeutic improved after regional delivery vial the lympahtics of subcutaneously delivered In^{111} labeled T101. Further studies included a comparison of quality imaging In^{131} T101 versus ^{111} T101. In this study, the In^{111} conjugate was clearly superior. This work now proceeds to further analysis of specificity of targeting by using an isotopically matched In^{111} T101-control antibody in sequential scanning studies with In^{111} T101. Information generated in the course of these studies include enhanced understanding of the pharmacology of antibody targeting, the immunogenicity of administered mouse immunoglobulin, and the efficiency if regional delivery techniques. These studies collectively provide the basis for proceeding with the radiolabeled T101 therapy trial which is discussed separately. Efforts have been productive, both in terms of published manuscripts and in developing useful collaborations with other investigators at the Clinical Center engaged in clinical research with monoclonal antibodies.

B. Small cell lung cancer has been extensively studied both at this Branch and elsewhere as a model of a neuroendocrine tumor. Small cell lung cancer has already been reported to produce over 25 different peptide hormone products. Recently, workers from our lab sequenced the gene for GRP from small cell lung cancer. A family of previously unknown peptides synthesized from open reading frames found on the GRP gene. Hetero anti-sera were developed to the three GRP gene associated peptides (G-Gap peptides). By several assays, immunologic evidence of expression of these three distinct products was documented in both small cell tumors and in fetal tissues. These facts suggest that despite the already known numbers of peptide products of small cell lung cancer, there may be a considerably larger number of small cell tumor products. With the rapid development of many areas of biotechnology, the techniques may now be available to begin systematically evaluating the total peptide synthetic capabilities of small cell.

C. We are interested in elucidating and additional new peptide products of small cell, we propose to focus on those peptide products that possess mitogenic capabilities. To facilitate this, we have invested considerable time in validating a semi-automatic colorimetric assay for evaluation of growth factor effects. The parameters to evaluation for such an application are considerably different than the conditions for the assay as reported by Carmichael and others from our Branch. The advantage of this assay is that it provides the exceedingly efficient assay to monitor for growth stimulatory effects, which will be essential when screening large numbers of purified fractions generated in typical HPLC purification efforts.

D. Using the semi-automatic colorimetric assay, we have already demonstrated the mitogenic effect of insulin-like growth factor-I (IGF-I) on small cell lung cancer cell lines. We have further demonstrated that this effect can be blocked by a monoclonal antibody specific for the anti-IGF-I receptor.

We have done considerable work in exploring the biology of IGF-I in small cell and it appears to be an attractive candidate to target for a therapy approach similar to the anti-GRP monoclonal antibody trial. In thinking about GRP and a candidate for immunotherapy, the limited role this molecule plays in normal adult physiology potentially permits one to completely block this peptide effect without lethal consequences. The situation with IGF-I may not be similar as this molecule plays a more obvious role in normal adult physiology. Although that might not prevent us from exploring the same type of anti-autocrine factor strategy we employed in the anti-GRP trial, it did provoke us to consider approaches.

E. Many investigators have suggested that cancer can be thought of as a reexpression of normal embryonic and fetal developmental processes. An exptrapolation is that autocrine type stimulation may be an important developmental mechanism. If so, such autocrine proliferation should be controlled through some signaling mechanism to allow for the uniform development of a fetus. In cancer, autocrine proliferation proceeds unabated either because of the regulatory signal. We tested to see if the stimulation of small cell lung cancer mediated be IGF-I could be inhibited by glucagon, a normal antagonist of IGF-I activity. Of interest, at a concentration of 10mg/ml, glucagon inhibits the growth enhancement of exogenous effect of IGF-I in other cell lines. In addition, we are attempting to define the mechanism mediating the inhibitory effect in the cell lines responsive to glucagon.

Significance to Biomedical Research and the Program of the Institute:

These studies have two goals: First to complete the ongoing trial which represent a first effort to establish the clinical utility of monoclonal antibody based imaging and treatment approaches; Second, we have design ongoing in vitro analysis in conjunction with the clinical trials as well as other laboratory investigations to develop second generation biological trials which lend to more effective therapeutic control of malignant proliferation.

Proposed Course:

The Phase I component of the anti-GRP trial is ongoing and will be extended to Phase II when appropriate. Clinical trials with the other antibodies will also continue with the goal of moving to radionuclide conjugate therapy using monoclonal antibodies in cutaneous T-cell lymphoma and lung cancer. Further work will continue to develop a feasible approach to block IGF-I stimulation of lung cancer. Dr. Cuttitta is generating antibodies against synthetic peptides from various portions of prepro IGF-I and the IGF-I receptor. Using either an available reagent or Branch derived product, we will do further in vivo work to block IGF-I stimulation. This work may lead us to a clinical trial in a similar fashion to the anti-GRP monoclonal antibody trial. Ultimately, one may choose to block both GRP and IGF-I stimulation. Careful analysis of early growth factor therapy trials may provide insight as to how best to proceed.

Publications:

1. Doyle LA, Cuttitta F, Mulshine JL, Bunn PA, Minna JD. Markedly different antibody repsonses to immunized small cell and non-small cell lung cancer cells. Cancer Res 1987;47:5009-5013.

2. Minna JD, Cuttitta F, Battey J, Mulshine J, Linnoila I, Gazdar AF, Trepel J, Sausville EA. Autocrine growth factors including gastrin releasing peptide (bombesin) as targets in the pathogenesis and treatment of lung cancer. In: DeVita V, Hellman S, Rosenberg S, eds. Important Advances in Oncology. Philadelphia: JB Lippincott Co, in press.

3. Nakanishi Y, Cuttitta F, Kasprzyk PG, Treston AM, Avis I, Minna JD, Kleinman HK, Mulshine JL. The effects of growth factors on the in vitro growth of small cell lung cancer as determined in a colorimetric assay. In: Rosen, Mulshine, Cuttitta, Abrams, eds. Tumor Biology. New York: Marcel Dekker, in press.

4. Nakanishi Y, Cuttitta F, Kasprzyk PG, Avis I, Steinbergt SM, Gazdar AF, Mulshine JL. Growth factor effects on small cell lung cancer cells using a colorimetric assay: Can a transferrin-like factor mediate autocrine growth. Exper Cell Biol, in press.

5. Nakanishi Y, Mulshine J, Kasprzyk P, Natale RB, Maneckjee R, Avis I, Treston AM, Gazdar AF, Minna JD, Cuttitta F. Small cell lung cancer cells autostimulate their growth via an insulin-like growth factor-I activity: evaluation of 4 cell lines. J Clin Invest, in press.

6. Treston AM, Kasprzyk PG, Covey T, Lee ED, Henion J, Yergery A, Cuttitta F, Mulshine JL. Applications of mass spectrometric identification and sequencing of novel neuro-peptides in lung cancer. In: Rosen, Mulshine, Cuttitta, Abrams, eds. The Biology of Cancer. New York: Marcel Dekker, in press.

7. Kasprzyk PG, Cuttitta, Treston AM, Avis I, Nakanishi Y, Wong H, Walsh J, Mulshine JL. Chemical considerations of solid-phase matrix interactions leads to improved quantitation of neuropeptides proceddings of the first international sypmosium of bombesin-like peptides. NY Acad Sci, in press.

8. Kasprzyk PG, Cuttitta F, Avis I, Nakanishi Y, Treston A, Wong H, Walsh JH, Mulshine JL. Solid phase peptide quantitation assay using labelled monoclonal antibody and gluteraldehyde fixation. Anal Biochem, in press.

9. Tockman MS, Gupta PK, Myers JD, Frost JK, Baylin SB, Gold EB, Mulshine JL. Sensitve and specific monoclonal antibody recognition of human lung cancer antigen on preserved suptum cells: a new approach to early lung cancer detection. J Clin Oncol, in press.

10. Mulshine JL, Kasprzyk PG, Nakanishi Y, Avis I, Seifter E, Cuttitta F. Considerations in the development of clinical trials employing monoclonal antibodies. Proceedings First International Workshop Small Cell Lung Cancer Antigens, in press.

11. Mulshine JL, Avis I, Treston AM, Moblely C, Kasprzyk PG, Carrasquillo JA, Larson SM, Nakanishi Y, Merchant B, Minna JD, Cuttitta F. Clinical use of a monoclonal antibody to bombesin-like peptide in patients with lung cancer. Proceedings of the First International Symposium of Bombesin-Like Peptides. NY Acad Sci, in press.

12. Mulshine J, Avis I, Treston AM, Kasprzyk, Nakanishi Y, Mobley C, Carrasquillo JA, Larson SM, Merchant B, Cuttitta F. In vivo diagnosis and therapy of human tumors with monoclonal antibodies selection of antibodies and preliminary clinical studies in small cell carcinoma of the lung. Nucl Med Biol, in press.

13. Rosen S, Mulshine JL, Cuttitta F, Abrams P. eds. Lung Cancer Biology. New York: Marcel Dekker, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06599-02 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Supportive Care Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barnett S. Kramer, MD	Senior Investigator	NCI-NMOB
Others:	Stephen Veach, MD	Capt., MC, USN	NCI-NMOB
	Michael Bolger, MD	Lt.Cdr., MD, USN	NCI-NMOB

COOPERATING UNITS *(if any)*

Division of Infectious Diseases, Naval Hospital, Bethesda (Walter Carney, Kenneth Wagner, Matthew Pollack); Pediatric Oncology Branch (Philip Pizzo)

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Clinical Investigations (Section Head: Daniel Ihde)

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this group is to investigate the prophylaxis and management of infectious complications of cancer patients undergoing chemotherapy. A recently completed study was one of prophylaxis of infection in neutropenic patients with passive immunization. It was a randomized study of intravenous gammaglobulin (IVIG) versus placebo. Preliminary analysis in 75 patients randomized at the NCI and Naval Hospital, Bethesda, showed no differences between IVIG and placebo in incidence of fever, documented infection, or duration of fever. The present project is a randomized study of monotherapy (ceftazidime) versus ceftazidime plus vancomycin for the initial empiric therapy of febrile cancer patients with very low white blood counts. To date, 85 episodes have been analyzed between the two collaborating centers (NCI-NMOB and University of Florida; 20 episodes at NCI-NMOB).; Early results show a somewhat better initial response rate to combined therapy, but a very similar ultimate outcome in survival. The randomized study of ceftazidime ± vancomycin is in progress. An early analysis of 85 episodes shows a higher initial response to the two drug regimen, but a similar ultimate outcome.

PROJECT DESCRIPTION

Supportive Care Project

Professional Staff:

PI:	Barnett S. Kramer, MD	Senior Investigator	NCI-NMOB
Others:	Stephen Veach, MD	Capt., MC, USN	NCI-NMOB
	Michael Bolger, MD	Lt.Cdr., MD, USN	NCI-NMOB

Collaborating Branches:

Division of Infectious Diseases, Naval Hospital, Bethesda (Walter Carney, Kenneth Wagner, Matthew Pollack); Pediatric Oncology Branch (Philip Pizzo)

Objectives:

1. IVIG Study (Completed since last annual report)

   a. To examine in a randomized double blinded study whether passive immunization with intravenous gamma globulin (IVIG) is effective as prophylaxis against infection in neutropenic cancer patients.

   b. To determine the pharmacokinetics of IVIG and correlate with efficacy of IVIG.

   c. To monitor toxicity of IVIG.

2. Ceftazidime Study

   To establish criteria for the addition of vancomycin to initial empiric monotherapy with ceftazidime in the empiric treatment of febrile neutropenic cancer patients.

Methods Employed:

Both of the above studies are prospective and randomized. The IVIG study was double-blinded with a placebo arm using albumin.

Assays for blood immunoglobulin levels are being performed.

Major Findings:

The IVIG Study is completed. An analysis of 75 episodes showed no differences in outcome (incidence of fever, incidence of infection, duration of fever) between IVIG and placebo.

Significance to Biomedical Research and the Program of the Institute:

Infection is a major cause of death in cancer patients and is a very common reason for hospitalization. The best therapeutic regimen for febrile neutropenic patients has not yet been established. We are looking for an effective non-toxic regimen.

Proposed Course:

We are presently negotiating with a manufacturer of GM-CSF (granulocyte-monocyte colony stimulating factor) to do a randomized study in acute leukemic patients who become febrile and neutropenic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07250-02 NMOB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

New Drug Discovery Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barnett S. Kramer, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Bruce Johnson, MD	Investigator	NCI-NMOB
	Daniel Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Mary Pat Dearing, MD	Medical Staff Fellow	NCI-NMOB/NCI-IDB

COOPERATING UNITS *(if any)*

Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Frederick Cancer Research Program (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian)

LAB/BRANCH

NCI-Navy Medical Oncology Branch

SECTION

Clinical Investigations (Section Head: Daniel Ihde)

INSTITUTE AND LOCATION

NCI, DCT, COP, Naval Hospital, Bethesda, MD 20814

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this group is to identify new agents of potential clinical use in treating solid tumors. A major effort over the past year has been the use of an in vitro assay which may be helpful as a preclinical screening model for antitumor agents. The model has been used to predict the clinical activity of 7 chemotherapeutic agents against 11 human colorectal carcinoma cell lines which have been developed in this branch. Using the model, we have shown that leucovorin enhances the in vitro cytotoxicity of the fluoropyridines versus our panel of colorectal cell lines. We are also presently doing a study to detect possible synergy between etoposide and cisplatin in a panel of 8 human bronchogenic carcinoma cell lines.

At present, we are involved in several trials of new experimental therapeutic agents: Dihydrolenperone in lung cancer; a radiolabeled monoclonal antibody (^{90}yttrium-T101) in mycosis fungoides and chronic lymphocytic leukemia. We also plan a trial of 4-ipomeanol in lung cancer and a Phase I trial of sulfamic acid.

PROJECT DESCRIPTION

New Drug Discovery Project

Professional Staff:

PI:	Barnett S. Kramer, MD	Senior Investigator	NCI-NMOB
Others:	Adi Gazdar, MD	Senior Investigator	NCI-NMOB
	Bruce Johnson, MD	Investigator	NCI-NMOB
	Daniel Ihde, MD	Senior Investigator	NCI-NMOB
	James Mulshine, MD	Senior Investigator	NCI-NMOB
	Mary Pat Dearing, MD	Medical Staff Fellow	NCI-NMOB/NCI-IDB

Collaborating Branches:

Radiation Oncology Branch (E. Glatstein); Nuclear Medicine, Clinical Center (J. Carrasquillo); Frederick Cancer Research Program (J. Mayo); Southern Research Institute (W.R. Laster); Investigational Drug Branch, CTEP (M. Christian)

Objectives:

1. Identification of new compounds for the treatment of solid tumors, especially colorectal carcinoma.

2. Preclinical testing of combinations of drugs to detect synergy.

3. Validation of in vitro chemosensitivity test.

4. Testing new compounds in the clinic for lung and colon cancers.

Methods Employed:

1. In vitro chemosensitivity: MTT assay (a tetrazolium-based colorimetric test for cell viability.

2. Phase I trials of new drugs in cancer (for example, dihydrolenperone and ipomeanol in lung cancer; sulfamic acid).

Major Findings:

1. 5-FU was the only one of 7 drugs tested which we predict would be effective in some of our colorectal cell lines.

2. Leucovorin enhanced the cytotoxicity of 5-FU and of FUDR in 10 of 11 colorectal cell lines tested.

3. The dihydrolenperone study is proceeding (see Dr. Bruce Johnson's Annual Report).

4. The ipomeanol study has not yet opened, nor has the ^{90}yttrium-T101 study.

Significance to Biomedical Research and the Program of the Institute:

New drug development is a major charge of the National Cancer Institute. The preclinical screening program of the NCI is based upon the MTT assay. It is important to pursue innovative therapies, such as treatment with radiolabeled monoclonal antibodies directed against malignant cells (e.g. ^{90}yttrium-T101).

Publications:

1. Park J-G, Kramer BS, Steinberg SM, et al. Chemosensitivity testing of human colorectal carcinoma cell lines using a tetrazolium-based colorimetric (MTT) assay. Cancer Res 1987;47:5875-5879.

2. Park J-G, Collins J, Gazdar A, Allegra C, Steinberg S, Green R, Kramer BS. Enhancement of fluorinated pyrimidine-induced cytotoxicity by leucovorin (LV) in human colorectal carcinoma cell lines (HCCL). Proc Am Soc Clin Oncol 1988;7:97.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06813-06 PB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Biology of Pediatric Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mark A. Israel	Head, Mol. Gene. Sec.	PB, NCI
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	C. Thiele	Senior Staff Fellow	PB, NCI
	L. Helman	Senior Staff Fellow	PB, NCI
	O. El-Badry	Guest Researcher	PB, NCI
	M. Cooper	Biotechnology Fellow	MB, NCI
	R. LaRocca	Medical Staff Fellow	MB, NCI
	T. Kidowaki	Guest Researcher	

COOPERATING UNITS (if any) University of PA (R. Packard, A. Evans, B. Emmanuel, J. Chatten: Johns Hopkins University (M. Levine, G. Hutchins); Sloan Kettering Memorial Hospital (D. Kelson); UCSF (M. Rosenblum); University of Upsalla (B. Westermark); Rockefeller University (J. Friedman)

LAB/BRANCH
Pediatric Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have used human neuroblastoma as a model system to study the molecular events associated with the development of malignant tumors during childhood. Recent work has indicated that among histologically-indistinguishable solid tumors there are different genetic entities which correspond to cells at different stages in the differentiation of the tissue in which solid tumors arise. Such a schema has been hypothesized for both lymphoid and hematopoietic malignancies in which specific markers of differentiation have been extensively characterized, but tumors of solid tissues have previously been classified largely in terms of the organ in which they arise. Our current focus is to use recombinant DNA technology to identify and characterize tissue specific markers, to use these markers to develop clinically efficacious tumor nosologies, and to study the molecular mechanisms important for the regulation of tissue-specific differentiation and the arrest of growth which invariably accompanies it. Ongoing work in this area suggests that the molecular mechanisms that give rise to different tumor subgroups we have been able to identify are pathologic alterations in regulatory pathways that function during specific times in normal development.

During the past year we have expanded our studies in this area and sought profiles of gene expression that distinguish among tumors occurring at several different organ sites. In this manner we hope to subgroup a number of tumor types into biologically homogeneous groups. Ultimately, our goal is to use this information to better classify patients for therapeutic trials and to develop novel therapeutic approaches based on the specific alterations underlying the development of individual malignancies.

Accomplishments and Results:

1. Evaluation of neuroblastoma tumor cell differentiation

To study the molecular mechanisms by which the in vitro growth arrest and differentiation of neuroblastoma tumor (NB) cell lines are mediated, we have evaluated the expression of genes uniquely expressed in neuronal cells and approximately twenty proto-oncogenes. Recently completed studies have focused on retinoic acid (RA) and cyclic AMP induced differentiation and have identified a series of genes that we are using as markers to study the altered differentiation that marks these cells. In addition, we have initiated a study to evaluate the effects of unregulated N-myc expression on the control of growth and differentiation of NB cells utilizing gene transfer technology. In these experiments, we determined that N-myc transfected NB cell lines are growth arrested by RA, yet cell lines expressing high levels of the transfected N-myc gene fail to morphologically differentiate suggesting that the regulation of N-myc expression is a critical event mediating the morphological differentiation of human neuroblastoma cell lines in response to RA.

2. Evaluation of neuroendocrine differentiation

In an effort to develop novel molecular probes that could be used as markers of neural crest differentiation, we have isolated and characterized several neuroendocrine specific cDNA clones. In the course of these experiments, we identified one molecular clone that encoded chromogranin A in its entirety and a second that encoded human preproneuropeptide Y (NPY), a neuroregulatory peptide found throughout the sympathetic nervous system. More recently, we have begun to characterize the cis acting regulatory sequences that might be important in mediating molecular events critical for neuroendocrine maturation. We have also studied the regulation of these genes in the developing human adrenal gland using in situ techniques and surveyed extensively their expression in normal and neoplastic neuroendocrine tissues. We found that while NPY was invariably expressed in benign human pheochromocytoma tumors, over 50% of malignant tumors do not express this gene.

3. Effect of extracellular matrix proteins on neuroblastoma tumor cell differentiation

We evaluated the effect of ECM proteins on NB cells in vitro because these proteins may be important during the migration and differentiation of neural crest cells during development. Our results indicate that several ECM proteins can promote morphologic differentiation but not growth arrest of NB cells. The growth and morphologic responses of NB cells to ECMs are similar to cAMP induced changes yet distinct from those observed with RA. Current studies are aimed at evaluating whether ECMs are critical in the response of NB cells to either of these biological response modifiers. While investigating the response of NB cells to laminin, we determined that NB cell lines expressing high levels of N-myc had a higher level of laminin receptor gene (LN-R) expression than those without N-myc expression. Decreased expression of both N-myc and LN-R occurred after the treatment of cells with RA. We are currently studying the relationship between these two genes by examining their expression in NB cell lines in which we have increased the N-myc gene copy number by DNA transfection.

4. Lineage analysis of human neuroblastomas

We have examined the relationship of normal adrenal medullary development and neuroblastoma tumors by 1) identifying a panel of developmentally-regulated, lineage-related genes of the human adrenal gland; 2) mapping out the temporal expression of these genes during normal human adrenal gland development using in-situ hybridization and immunohistochemistry; and 3) examining pathologic specimens to determine the pattern of expression of these genes in tumors. We found that NB cell lines and tumor specimens correspond to developmental stages which can be recognized during normal neural crest maturation. We believe these subgroups of NB to be analogous to the subgroup of lymphoma that correspond to developmental stages of normal lymphocyte maturation. We are now pursuing the possibility that factors which normally regulate the proliferation and differentiation of fetal adrenal neuroblasts may be useful in modifying the malignant behavior of neuroblastoma tumors.

5. The role of growth factors in the proliferation of neuroblastoma tumor cells

Using a ^{3}H-thymidine incorporation bioassay, we determined that exogenously added IGF-I, IGF-II, or insulin stimulated the growth of all four NB cell lines and one neuroepithelioma cell line that we evaluated. In a series of additional studies, we found that IGF-II is an autocrine growth stimulator of one human NB cell line. Subsequently we found that many NB cell lines and tumors, as well as pheochromocytoma tumors, express the genes encoding both IGF-II and the IGF-I receptor. These finding suggest that IGF-II may play a role in the autocrine growth of these several different tumors arising in the adrenal gland.

6. Pattern of proto-oncogene expression in human glioma cell lines

We have analyzed the pattern of proto-oncogene expression in over thirty cell lines derived from biopsy specimens of patients with malignant gliomas to determine whether it was possible to recognize discrete, biologically homogenous entities. Our results indicate the constant or near constant expression of c-myc, neu, raf/mil, EGF-R and fos, undetectable expression of N-myc and L-myc, and heterogenous expression of c-sis and myb in these tumor cell lines. C-sis expression was detected only in cells with a pleomorphic-glial or epithelial morphology, and GFAP expression was only found in the subgroup of these tumor cell lines with a pleomorphic-glial morphology. Comparable findings suggesting that proto-oncogenes are differentially expressed in biologically relevant subgroups of colon carcinoma cell lines is also emerging from other ongoing studies in our laboratory. We are currently pursuing the implications of these findings for the development of a tumor nosology for gliomas that reflects cellular features of potential therapeutic importance.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06830-18 PB

PERIOD COVERED

October 1, 1987 - September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Infectious Complications of Malignancy: Diagnosis, Management and Prevention

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Philip A. Pizzo	Head, Infectious Disease Section, Chief	PB, NCI
Other:	J. Falloon	Senior Staff Fellow	PB, NCI
	E. Albano	Special Volunteer (Children's Hospital, DC)	PB, NCI
	M. Rubin	Special Volunteer (Cornell University)	PB, NCI
	T. Walsh	Medical Officer	PB, NCI
	D. Callender	Medical Staff Fellow	MB, NCI

Continued on next page

COOPERATING UNITS (if any)

Medicine Branch, Surgery Branch, NCI; Diagnostic Microbiology, CC; Bethesda Naval Hospital; University of Miami; Duke University

LAB/BRANCH

Pediatric Branch

SECTION

Infectious Disease

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	4.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Our studies are devoted to developing methods to define cancer patients who are at risk for developing serious infection, to improving the ability to diagnose these infections early, to treat them effectively, and ultimately to prevent them. We are developing new therapeutic approaches based on the availability of new antibiotics, particularly the beta-lactams and the quinolones. We have shown that certain beta-lactams used as single agents can replace the need for combination antibiotic therapy. Our studies are also defining the appropriate antibiotic therapy for documented infections, particularly the role of oral antibiotic therapy; the necessary duration of empiric therapy for patients with unexplained fevers and the choice of empiric antifungal therapy. In addition, we are studying the best type of indwelling catheter to use in the cancer patient and how to manage their related side effects.

We have developed a unique model for studying the pathophysiology, natural history, treatment and prevention of invasive candidiasis in the neutropenic host. This model permits the testing of new antifungal agents as well as immunoregulatory agents. To prevent infections we are evaluating the role of passive immunization with a pooled immunoglobulin preparation that contains activity against the Enterobacteriaceae as well as the pseudomonads. We are also studying other immunoregulatory agents that may serve as adjuncts to the treatment of infection, including interleukin 1 and 2, GM-CSF, G-CSF and M-CSF.

We have completed Phase I trials with the anti-retroviral agent, azidothymidine for children with AIDS or ARC and have initiated Phase I trials with DDC and with the combination of AZT plus GM-CSF.

Professional Personnel (Continued):

J. Eddy	Nurse Specialist (Research)	PB, NCI
J. Gress	Guest Researcher	PB, NCI
D. Marshall	Nurse Specialist (Research)	PB, NCI
M. Einloth	Nurse Specialist (Research)	PB, NCI

Clinical and Laboratory Studies

1. We are continuing a prospective randomized trial comparing monotherapy with a third-generation cephalosporin (ceftazidime) to a carbapenem antibiotic (imipenem) for the empiric management of febrile neutropenic patients. This trial demonstrates that monotherapy is successful for the initial empiric management of patients who become febrile while neutropenic, an observation which suggests that synergistic therapy is not necessary if a single agent provides broad-spectrum activity and high serum bactericidal levels. This offers new approaches to therapy that are both practical and cost effective.

   To date, we have randomized 246 episodes of fever and neutropenia, 122 to imipenem and 124 to ceftazidime. Both groups are comparable in the time to defervescence and success in treating patients with defined infections or with unexplained fever. This study is continuing.

2. Our studies of empiric antibiotic therapy have also placed in perspective the appropriate utilization for the aminoglycosides and vancomycin, pointing out that although helpful, these agents are really only necessary in approximately 15% of cancer patients who become febrile and neutropenic.

3. In a randomized trial, we have demonstrated that an oral imidazole, ketoconazole, is as effective as amphotericin B in preventing the emergence of systemic fungal infections in cancer patients who remain persistently febrile while receiving broad-spectrum antibiotic therapy. Studies to evaluate a new azole antifungal agent, fluconazole, are being initiated.

4. In a randomized trial, we have demonstrated that it is appropriate to continue empiric antibiotic therapy for a limited (i.e., 2 week) course for patients who have defervesced following the initiation of antibiotics but who remain afebrile.

   In a follow-up study, we are comparing the use of a new class of oral antibiotics, the quinolines, for patients who have defervesced on parenteral therapy, have no defined site of infection, and remain persistently granulocytopenic. This study has considerable importance, since it can serve to re-define the role of inpatient versus outpatient therapy for treating the infectious complications that occur in tandem with cancer therapy. To date, 57 patients have been randomized.

5. We have demonstrated the importance of anaerobes in contributing to perianal cellulitis and of the early initiation of specific anti-anaerobic therapy in diminishing the progression of these infections and limiting the need for surgical intervention.

6. To decrease the frequency of infectious complications associated with indwelling intravenous catheters of the Hickman-Broviac type, we have performed a randomized trial to compare the Hickman-type catheter to a subcutaneously implanted catheter (Port-A-Cath). Since the subcutaneous catheter requires less manipulation, our hypothesis is that it will have a lower incidence of infection. However, if infected, it is possible that these infections will not be capable of eradication unless the catheter is removed. To date, 100 patients have been randomized, 52 to the Port-A-Cath, 48 to the Hickman catheters. The long term follow-up on this study is undergoing completion.

7. To reduce the incidence of infection in patients who have protracted neutropenia, we evaluated, in a double-blind randomized trial, the value of passive immunization with a pooled intravenous immunoglobulin (IVIG). We also assessed the utility of this immunoglobulin in attenuating the types of infections which occur. Of 65 evaluable episodes, 33 were randomized to IVIG and 32 to placebo. No benefit from the IVIG was observed.

8. We have developed a unique model of candidiasis that closely mimics the pattern of infection that occurs in patients with prolonged periods of neutropenia. This model utilizes rabbits that are maintained in a neutropenic state for 3-4 weeks. This model permits study of the pathophysiology and natural history of invasive fungal infection in a profoundly neutropenic host. It lends itself to the study of various therapeutic and preventative strategies as well as the role of immunoregulatory agents as adjuvants. This model is particularly important in view of the increasing prominance that fungal infections are playing in compromised hosts and the difficulty that exists in studying them. We have also developed models of fungal myositis, endophthalitis and catheter infections.

9. In our neutropenic rabbit model, we have domonstrated the unique pathological lesion that characterizes the syndrome of hepatic candidiasis. This syndrome has only been appreciated during the past several years and we have reviewed the cases that have presented to the NIH, compared them to the world's literature and compared them to the lesions that occur in the experimental model. This will provide insight into diagnosis and therapy and eventually, prevention.

10. In our neutropenic rabbit model, we have evaluated all the antifungal agents that are currently available, as single agents and in combination. We have shown that the kidney is the most difficult organ from which to eradicate infection. In this model, only the combination of amphotericin B plus 5-fluorocytosine is effective in eliminating infection. We have

also evaluated itraconazole and fluconazole as well as several other azoles in models of sublethal infection, lethal infection, hepatosplenic candidiasis and preventive therapy.

We are evaluating GM-CSF, IL-1, M-CSF as adjuncts to therapy in in vitro and in vivo fungal systems.

11. The incidence of infants acquiring HIV infection transplacentally is increasing and the population of pediatric AIDS is expanding. With the exception of immunoglobulins, therapeutic interventions have been limited in pediatric AIDS.

To achieve plasms and cerebrospinal fluid concentrations of azidothymidine (AZT) that might provide constant inhibition of HIV replication as well as overcome the difficulties associated with administering a drug having a short half life, we evaluated the continuous delivery of AZT via a Hickman-Broviac catheter with a portable Pancreatec programmable infusion pump. Twenty-one children with symptomatic HIV disease (CDC Class P-2), ranging in age from 14 months to 12 years were enrolled. HIV was acquired by transfusion in 11 (52%), from factor replacement for hemophilia in 2 (10%), and perinatally in 8 (38%). Prior to initiation of AZT, the mean CD4/CD8 ratio was 0.18 with 11 patients having CD4 counts $<200/mm^3$. Of note, 13/21 (62%) children entering this trial had evidence of neurodevelopmental abnormalities before beginning AZT.

Four dose levels were studied, and plasma and CSF concentrations of AZT were measured: Level 1, 0.5 mg/kg/h; Level 2, 0.9 mg/kg/h; Level 3, 1.4 mg/kg/h; Level 4, 1.8 mg/kg/h. Plasma concentrations achieved at the respective dose levels were 1.9±3μM; 2.8±4μM; 3.1±.1μM; and 4.5±.0μM. The steady state CSF:plasma ratio was 0.24±.07. Therapy was well tolerated and toxicity was limited to bone marrow suppression. Anemia (hemoglobin <8gm/dl) served as criteria for transfusion and was required in 13 patients. Dose-limiting neutropenia (<500 polymorphonuclear leukocytes/mm^3) occurred at doses 1.4mg/kg/d or above. On this basis, the recommended dosage is between 0.9 - 1.4mg/kg/d.

Dramatic improvement occurred in all 13 children presenting with encephalopathy prior to treatment. Serial IQ measurements before therapy and after 3 and 6 months of AZT showed a significant improvement ($p<0.005$) in the overall IQ score, and included improvement in both verbal IQ ($p<0.05$) and Performance IQ ($p<0.02$). Of note, 6 children without detectable evidence of encephalopathy prior to AZT also showed improvements ($p<0.01$) in IQ scores from baseline compared to 6 month follow-up, suggesting that cognitive impairment may be among the earliest manifestations of AIDS encephalopathy in children, underscoring the need for modalities that will treat the central nervous system, even in patients who do not appear to have neurodevelopmental deficits.

In addition to improvements of neurological function, patients have increases in appetite and weight, decreases in lymphadenopathy and hepatosplenomegaly, lowering of the immunoglobulin level and rises in CD4 cell number while receiving AZT. In some patients the improvements in encephalopathy occurred independent of other changes.

This study demonstrates that AZT is beneficial for children with symptomatic HIV infection, especially those who have or who are at risk for neuroencephalopathy.

In addition, we are collaborating with Duke and the University of Miami in performing an intermittent bolus infusion study with AZT. Thirty-five children have been entered into this trial of which we have enrolled sixteen patients. While improvement from neurodevelopmental delays were seen with oral intermittent therapy, the degree and consistency of improvement was significantly lower than when AZT was given by the continuous infusion route of delivery. To further evaluate whether a steady-state of AZT is better than intermittent concentrations, a randomized trial is being initiated.

12. In addition to AZT, we are conducting a Phase I evaluation of dideoxycytidine (DDC) in children with symptomatic HIV infection. To date, 5 children have been studied. Once completing the 8-week trial of DDC, children will be treated on an alternating schedule of DDC and AZT to determine whether efficacy can be maintained and toxicity lowered.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06840-13 PB

PERIOD COVERED
October 1, 1987, to September 30, 1988

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)
Treatment of Acute Leukemia

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	David G. Poplack	Head, Leukemia Biology Section	PB, NCI
Others:			
	F. Balis	Investigator	PB, NCI
	R. Heideman	Investigator	PB, NCI

COOPERATING UNITS (*if any*)
Medicine Branch, NCI (A. Fojo); Clin Pathology, NCI (L. Neckers); Navy, NCI (L. Kirsch); Children's Cancer Study Group (G. Reaman).

LAB/BRANCH
Pediatric Branch

SECTION
Leukemia Biology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type Do not exceed the space provided.*)

Clinical research into the biology and treatment of acute leukemia is pursued with particular emphasis on acute lymphoblastic leukemia (ALL) of childhood. Major issues being addressed include: 1) development of therapeutic strategies aimed at improving overall prognosis of children with ALL, 2) investigation into the mechanisms of treatment failure with particular emphasis on evaluation of pharmacologic approaches to leukemic therapy, and 3) characterization of adverse sequelae of antileukemic therapy and design of treatment regimens which avoid them.

An earlier ALL treatment protocol demonstrated that high-dose, protracted systemic methotrexate infusions could substitute for cranial radiation as central nervous system (CNS) preventive therapy for the majority of patients with ALL. Analysis of data from this study also identified a patient group at particular risk for CNS relapse. A new, high risk protocol has been devised in an attempt to improve the prognosis for these and other poor risk patients. The results to date indicate that this therapy is highly effective in preventing both systemic and central nervous system relapses while avoiding the use of cranial radiation. In patients in the average risk category, a comparison of two forms of CNS preventive therapy (intrathecal vs High Dose Methotrexate) is under way. A major, multi-institutional pharmacologic monitoring protocol is in progress which is studying the relationship between the bioavailability of orally administered maintenance chemotherapy and relapse in children with ALL. On the basis of in vitro studies indicating that Interleukin-2 will induce phenotypic and functional maturation in acute lymphoblastic leukemia cells, a Phase I study of this potential antileukemic approach is being instituted. A study demonstrating expression of the β IL-2 receptor on hematopoietic malignant cells offers a potential avenue for future therapy. Detailed analysis of the immunologic and molecular phenotype of acute lymphoblastic leukemia has led to the concept of a hierarchy of differentiation for both T cell and pre-B cell ALL.

Objectives

1. To develop effective therapeutic regimens in acute lymphoblastic leukemia which provide maximum tumor cell kill and improve the prognosis of children with acute lymphoblastic leukemia.

2. To study pharmacologic approaches to leukemia therapy in an attempt to probe the pharmacologic reasons for treatment failure in this disease.

3. To evaluate the short- and long-term effects of antileukemic therapy on growth, development and organ function, with particular reference to the central nervous system.

Methods and Major Findings:

A. Treatment Studies of Acute Lymphoblastic Leukemia

1. NCI 77-02/CCG 191 Treatment Protocol

A randomized protocol investigating the efficacy of high dose intravenous methotrexate infusions as CNS preventive therapy. Patients received either cranial radiation plus intrathecal methotrexate or high dose 24-hour intravenous methotrexate infusions. One-hundred-eighty-one (181) average and high risk patients were randomized on this study. The overall remission rate was 98%. The continuous complete remission rate is approximately 70% at three years for the entire study group. With a median duration on study of 76 months, there is no significant difference in the CNS relapse rate for either treatment group. Longitudinal evaluation of neuropsychological function has demonstrated a striking decrease in IQ test scores and impaired academic achievement in children treated with cranial radiation and intrathecal chemotherapy. No such changes have been observed in children treated with high dose methotrexate.

2. NCI 83-P/CCG 134P

The major aim of this pilot protocol is to demonstrate that high risk patients can be effectively treated on a regimen that uses CNS preventive therapy devoid of cranial radiation. To date, 75 patients have been entered on study; all achieved complete remission. With a median duration on study of 30 months, the event free survival (at 18 months) is approximately 80%. The occurrence of isolated CNS relapse in only one patient, to date, suggests that effective CNS preventive therapy can be achieved without the use of cranial radiation in high risk patients.

3. NCI 84-A/CCG 144

This protocol randomizes average risk patients to one of two forms of CNS preventive therapy - either high dose methotrexate infusions or intrathecal methotrexate alone. One hundred fifty patients have been randomized on study. With a median potential duration on study of 20 months, there is no significant difference in the CNS or bone marrow relapse rate in either treatment arm.

B. Pharmacologic Approaches to Leukemic Therapy: Relationship to Treatment Failure

A detailed study of the bioavailability of the major orally administered antileukemic agents is being undertaken in an attempt to examine the reasons for treatment failure in children with ALL. This study is attempting to correlate the results of prospective periodic pharmacokinetic bioavailability studies of 6-MP and methotrexate with relapse rate and remission duration in a multi-institutional setting. Approximately 90 patients have been entered to date.

A study of this multidrug resistance gene MDR-1 expression in lymphoblasts from patients with ALL demonstrated evidence of overexpression, without gene amplification, in approximatley 25% of a group of patients who either relapsed or failed primary induction therapy. This finding is leading to a new protocol to study the ability of selected calcium channel blocking agents to reverse the development of drug resistance in children with ALL.

C. Molecular Biology of Acute Lymphoblastic Leukemia

Collaborative studies are investigating the status of immunoglobulin gene rearrangement and T-cell receptor gene status in acute leukemic lymphoblasts. Studies to date have enabled us to construct a hierarchy of differentiation for both pre-B cell precursor ALL (by immunoglobulin gene rearrangement) and for T-cell rearrangement (using T-cell receptor gene rearrangement). Recent studies have examined δ T cell receptor gene rearrangement and correlated its presence with the stage of differentiation.

D. Interleukin-2 as potential therapy for Acute Lymphoblastic Leukemia

In vitro studies with IL-2 have demonstrated its ablity to induce phenotypic and functional maturation in a subset of acute leukemic lymphoblasts. This observation led to the development of a Phase I trial in patients with hematologic malignancies which is currently in progress. Demonstration of the existence of the β subunit of the IL-2 receptor on leukemia cells provides a mechanism for us to specifically kill cells using monoclonal antibodies specific for this receptor. Study of this approach is under way.

Publications

Balis FM, Holcenberg JS, Zimm S, Tubergen D, Collins JM, Murphy RF, Gilchrist G, Hammond D, Poplack DG. The effect of methotrexate on the bioavailability of oral 6-mercaptopurine, Clin Pharmacol Therapeu 1987; 41:384-7.

Felix CA, Wright JJ, Poplack DG, Reaman GH, Cole D, Goldman P, Korsmeyer SJ. T cell receptor α-, β-, and γ-genes in T cell and pre-B cell acute lymphoblastic leukemia, J Clin Invest 1987; 80:545-56.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06880-11 PB

PERIOD COVERED
October 1, 1987, to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Clinical Pharmacology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	David G. Poplack	Head, Leukemia Biology Section	PB, NCI
Others:			
	F. Balis	Investigator	PB, NCI
	R. Heideman	Investigator	PB, NCI

COOPERATING UNITS (if any)
Medicine Branch, NCI (C. Allegra); Childrens Cancer Study Group (J. Holcenberg)

LAB/BRANCH
Pediatric Branch

SECTION
Leukemia Biology Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The clinical pharmacology of antineoplastic agents used in the treatment of pediatric malignancies is studied with emphasis on the role of pharmacologic monitoring and on both pre-clinical and clinical pharmacologic studies of Phase I agents. The clinical pharmacology of orally administered antileukemic agents has been evaluated and the limited bioavailability and variable drug levels of 6-MP achieved following oral administration has been documented. Studies are underway to determine the extent to which this phenomenon is the cause of treatment failure. Additional efforts to optimize 6-MP administration have been based on in vitro studies which have demonstrated a need for prolonged exposure to cytocidal concentrations of drug to maximize leukemic cell kill. Clinical Phase II protocols evaluating prolonged intravenous 6-MP infusions for acute leukemia, brain tumors and solid tumors are under way. Preclinical and clinical pharmacokinetic studies of a variety of new agents, such as Fazarabine and Piritrexim are in progress. A Phase I study of Thiotepa in pediatric patients has been completed which documents the excellent penetration of this agent into the CNS. A major effort of this project is to study experimental approaches to the treatment of CNS malignancy. A unique primate model is utilized to study the CNS pharmacokinetics of various intrathecally and intravenously administered chemotherapeutic agents; to evaluate the neurotoxicities of various CNS treatments; and to evaluate and screen newer CNS treatment modalities and drug schedules. Information gained from the studies with this model is then applied to the design of clinical treatment protocols. Clinical studies of intrathecal AZQ and continuous intrathecal 6MP are in progress. Pre-clinical studies evaluating intra-CSF drug administration via indwelling drug delivery devices is under way. This model has also been used to assess the CNS penetration of a variety of new anti-retroviral agents (used to treat AIDS). Clinical pharmacologic support for ongoing Phase I trials in children with AIDS is also pursued in this section.

Objectives:

1. To perform pre-clinical and clinical pharmacologic studies on new agents with particular emphasis on those being used to treat pediatric malignancies and those with potential activity against CNS malignancies.

2. To explore a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.

3. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.

4. To perform pre-clinical and clinical pharmacologic evaluation of new anti-retroviral agents undergoing Phase I testing in children.

Methods Employed and Major Findings:

A. Clinical Pharmacology of Antineoplastic Agents

1. Studies with 6-Mercaptopurine

Extensive preclinical and pharmacokinetic studies of 6-mercaptopurine (see Project Numbers Z01-CM-06840-09-PB and Z01-CM-06880-08PB) led to the development of current Phase II Studies evaluating the efficacy of prolonged intravenous 6-MP infusions for 1) acute lymphoblastic leukemia, 2) brain tumors, and 3) pediatric solid tumors. These studies are in progress.

2. Clinical Studies of Thiotepa

We have evaluated the clinical pharmacology of Thiotepa in children with malignancy. Thiotepa is an active alkylating agent with a steeper dose response curve than cyclophosphamide. Our studies have demonstrated that substantial amounts of both thiotepa and its metabolite Tepa are present in the CNS following intravenous administration. This data indicates that this route of administration may be a more optimal one to approach CNS disease with this agent. As a result of these studies, a Phase I study of intravenous thiotepa in pediatric patients was undertaken and completed. Further, Phase II, studies of this compound in children with cancer have recently been initiated.

3. Phase I Studies

In addition to studies of Thiotepa we have performed Phase I studies on other agents including Fazarabine, Interleukin (IL-2) and Piritrexim.

4. Other Pre-Clincal Studies

We have evaluated the feasibility of intrathecal administration of 4-hydroperoxycyclophosphamide, a preactivated analog of cyclophosphamide, which does not require hepatic activation, and thus is potentially feasible for regional administration. We have evaluated the pharmacokinetics of 4-hydroperoxycyclophosphamide in the CSF following intraventricular administration. Our results indicate that therapeutic levels of this compound can be achieved in cerebrospinal fluid following intraventricular dosing. In addition we have demonstrated that intrathecal therapy with this compound is feasible and safe. Further studies of this approach are being pursued in an effort to introduce this approach into clinical studies as rapidly as possible.

Immunotoxins, antibodies linked to peptide toxins, represent new potent and specific drugs potentially useful for treatment of malignancies, such as T-cell administration of a cytotoxic anti T-cell ALL monoclonal antibody - Ricin-A Chain conjugate. The pharmacology and toxicity following intraventricular administration was assessed. The CSF clearance of the conjugate appears to be by CSF bulk flow. Laboratory studies indicate the CSF concentrations of immunoconjugate exceed the LD50 for T-ALL cells by 3 1/2 logs. Following intraventricular administration, a concentration exceeding the LD50 is maintained in the CSF for greater than 24 hours. No clinical neurotoxicity was observed in these studies. The significance of a mild eosinophilic pleocytosis is being evaluated. Based on these findings, clinical studies of this approach are being planned.

An innovative system which permits assessment of continuous intra-CSF drug administration has been developed in the sub-human primate. A unique lateral ventricular catheter attached to a continuous infusion portable pump, together with the 4th ventricular Ommaya reservoir enables us to deliver and monitor drug administration by continuous intra-CSF administration. Interim results with methotrexate demonstrate substantial advantages for this approach.

5. Pre-clinical and Clinical Pharmacologic Studies of Anti-Retroviral Agents.

We have performed pre-clincal and clinical pharmacologic studies with a variety of anti-AIDS agents being studied on our service. These include studies of AZT, DDC and DDA. They are detailed in section Z01 CM 06830-18 PB of this report.

6. Phase I/II of Intrathecal AZQ

Based on work in our subhuman primate model, a Phase I/II Study of Intrathecal AZQ has been conducted and is still in progress. Results to date have been encouraging. A total of 21 patients have been treated. There have been 9 complete responses and 7 partial responses of the 17 evaluable patients. This agent obviously has demonstrated activity against malignant meningeal disease.

7. Phase I Study of Intrathecal 6-MP

Based on studies performed in our subhuman primate model, we have demonstrated the feasibility of intrathecal administration of 6-mercaptopurine. A Phase I Study has been initiated. Of the 8 patients entered on study to date, 2 have achieved a complete response.

Publications

Balis FM, Lester CM, Chrousos G, Heideman RL, Poplack DG. Differences in cerebrospinal fluid penetration of corticosteroids: possible relationship to the prevention of meningeal leukemia, J Clin Oncol 1987; 5:202-7.

Winick N, Kamen B, Balis F, Holcenberg J, Lester C, Poplack D. Folate and methotrexate polyglutamate tissue levels in Rhesus monkeys following chronic low-dose methotrexate, Cancer Drug Del 1987; 4(1):25-31.

Balis FM, Patel R, Luks E, Doherty K, Holcenberg J, Tan C, Reaman GH, Belasco J, Ettinger LJ, Zimm S, Poplack DG. Pediatric phase I trial and pharmacokinetic study of trimetrexate (NSC 352122), Cancer Res 1987; 47:4973-6.

Arndt CAS, Colvin M, Balis F, Lester C, Johnson G, Poplack DG. Intrathecal administration of 4-hydroperoxycyclophosphamide in rhesus monkeys, Cancer Res 1987; 47:5932-4.

Murphy RF, Balis FM, Poplack DG. Stability of 5-Fluorouracil in whole blood and plasma, Clin Chem 1987; 33(12):1199-2000.

Arndt CAS, Walsh TJ, McCully CL, Balis FM, Pizzo PA, Poplack DG. Fluconazole penetration into cerebrospinal fluid: implications for treating fungal infections of the central nervous system, J Infect Dis, 1988; 157(1):178-80.

Collins JM, Klecker RW Jr, Kelley JA, Roth JS, McCully CL, Balis FM, Poplack DG. Pyrimidine deoxynucleosides: selectivity of penetration into cerebrospinal fluid, J Pharmacol Exper Ther 1988; 245(2):466-70.

Greene RF, Miser A, Lester CM, Balis FM, Poplack DG. Cerebrospinal fluid and plasma pharmacokinetics of morphine infusions in patients and rhesus monkeys, Pain 1987; 30:339-48.

Kelley JA, Litterst CL, Roth JS, Vistica DT, Poplack DG, Cooney DA, Nadkarni M, Balis FM, Broder S, Johns DG. The disposition and metabolism of 2',3'-dideoxycytidine, an in vitro inhibitor of HTLV-III infectivity, in mice and monkeys, Drug Metabolism Disp 1987; 15(5):595-601.

Rogers P, Allegra CJ, Murphy RF, Drake JC, Masur H, Poplack DG, Chabner BA, Parrillo JE, Lane C, Balis F. Bioavailability of oral trimetrexate in patients with acquired immunodeficiency syndrome, Antimicrob Agents Chemother, 1988; 32(3):324-6.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06890-09 PB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Lymphoma Biology and Epstein Barr Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ian Magrath	Head, Lymphoma Biology Section	PB, NCI
Others:	Francisco Barriga	Clinical Associate	PB, NCI
	Bruce Shiramizu	Clinical Associate	PB, NCI
	Mary McManaway	Biotechnology Fellow	PB, NCI
	Melissa Adde	Clinical Nurse Specialist	PB, NCI
	Ronald Liebermann	Senior Investigator	FDA
	Leonard Neckers	Senior Investigator	LP, NCI

COOPERATING UNITS *(if any)*

Department of Pathology, New York University (r. Dalla-Favera)

LAB/BRANCH

Pediatric Branch

SECTION

Lymphoma Biology Section

INSTITUTE AND LOCATION

National Cancer Institute, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	4.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Research into the biology and treatment of pediatric lymphomas is directed towards the development of improved treatment strategies, and has both short term and long term goals. The primary short term goals include increasing the therapeutic index of combination chemotherapy regimens and continuing to characterize prognostic factors and patterns of disease relevant to treatment approaches. The primary long term goal is to develop a detailed understanding of the pathogenetic mechanisms of the non-Hodgkin's lymphomas, with particular emphasis on the small cleaved lymphomas, with the ultimate goal of utilizing this information in the development of novel treatment approaches.

105 patients with non-Hodgkin's lymphoma have been treated according to PB protocol 77-C-145. This protocol has produced a wealth of information regarding prognostic factors, patterns of disease, and the apparent lack of a role for radiation therapy in all patients except, possibly, those presenting with testicular lymphoma. In addition, tumor samples collected from patients treated with this protocol have provided a unique library of cell lines which have been invaluable both to ourselves and to many other laboratories in the study of the biology and molecular genetics of the small non-cleaved lymphomas. Ongoing studies are directed towards the identification of the pathogenetic role of EBV, the pathogenetic and clinical significance of chromosomal breakpoint locations, and attempts to develop a means of specifically inhibiting the expression of the c-myc oncogene in Burkitt's lymphoma.

Pilot protocols have provided toxicity information on a modified and considerably curtailed version of protocol 77-C-145 and identified a new, effective drug combination now ready to be incorporated into primary therapy.

CLINICAL AND LABORATORY STUDIES

Clinical Protocol NCI-77-C-145

This protocol remains open only for patients with lymphoblastic lymphomas and patients with limited stage, small non-cleaved lymphomas. With more than 100 patients so far entered on study, it provides an important resource for clinical information. Recently, we have completed analyses of the associated disease patterns and relevance to treatment of testicular and bony sites of tumor. In addition, we have demonstrated that multiple bone marrow sampling is as important in lymphomas in young patients as it is in adults -a finding which goes against accepted (but not well documented) dogma. At present we are initiating a dose intensity analysis in order to determine whether such an analysis can provide data regarding the relative efficacy of individual components of the combination regimen, and the overall importance, to the outcome of treatment, of dose intensity. We have also compared the results of protocol 77-C-145 with those of the more recently initiated Total B protocol of St Jude, in which some 80% of patients achieve long term survival. When patient groups with similar levels of serum LDH were compared, survival curves were essentially identical.

Pilot Protocol 85-C-67 for Patients with Extensive Non-Lymphoblastic Lymphoma

To date 7 patients have been entered into this protocol. All had extensive disease, 4 with diffuse bone marrow involvement. Six patients achieved complete remission, and 3 patients are alive and disease free for more than 1 year. Three patients have died from recurrent disease and one patient is currently undergoing therapy for relapse. The primary purpose of this protocol has been served, since it is clear that even patients with extremely extensive disease can achieve long term survival with only 6 cycles of therapy. In addition, toxicity has proved to be tolerable apart from, surprisingly, vincristine neurotoxicity. Five of the 7 patients experienced severe vincristine-induced neuropathy. This protocol, a modified version of protocol 77-C-145, will serve as one component of the new primary protocol for the treatment of extensive small non-cleaved lymphomas. The dose rate of vincristine will be suitably modified.

Pilot Protocol 85-C-62 for Patients with Recurrent Non-Lymphoblastic Lymphoma

To date 12 patients with recurrent non-lymphoblastic lymphoma have been entered into this protocol. Two of the 10 patients evaluable at present (two are still undergoing their first therapy cycle) achieved complete remission and 7 obtained a partial response. There was a single patient who failed to achieve a partial response. These results are gratifying in a patient group with an exceedingly poor prognosis, all except one having failed their previous treatment protocol "on therapy." Thus, this protocol has demonstrated that the combination of high dose ara-C, VP-16 and ifosfamide is active, even after failure of protocol 85-C-67. This finding justifies the planned incorporation of protocol 85-C-62 into the schema of the new front-line protocol for patients with extensive non-lymphoblastic lymphomas. The latter will consist of a regimen alternating between the two non-cross-resistant but active drug combinations currently existing as pilot protocols.

Molecular Analysis of Chromosomal Breakpoint Locations

Non-random chromosomal translocations, the most important being an 8;14 translocation, provide a critical element in the pathogenesis of the small non-cleaved lymphomas. Our studies of the significance of the breakpoint locations on chromosomes 8 and 14 have revealed much interesting information. We have previously described a difference in this regard between endemic and sporadic Burkitt's lymphomas, and have now extended these observations to a more precise analysis of the breakpoint locations in relationship to the c-myc allele involved in the translocation. We have demonstrated that chromosomal breaks occur in the first intron of the c-myc gene in some 50% of sporadic tumors, but not at all, to date, in endemic tumors. These findings strongly suggest that the mechanism of deregulation of c-myc differs in endemic and sporadic cases. In endemic tumors the breakpoint is nearly always far 5' of the gene, but mutations can invariably be found in the first exon of c-myc. A possible means of deregulation of c-myc in endemic tumors is, therefore, the abrogation of the elongation block present at the 3' end of the first exon and known to be an important method of regulation of c-myc transcription. In tumors with an intron break, transcripts begin from a cryptic promoter in the intron itself - i.e. the block to transcription is bypassed and presumably another factor becomes operative in order to cause the initiation of transcripts from the cryptic promoter. We have also described one cell line in which an immediate 5' breakpoint results in altered promoter utilization, due to its effect on the ability of the regulatory elements in this 5' region to influence the expression of c-myc. The issue of the relevance of breakpoint locations to the mechanism of c-myc deregulation is the subject of ongoing studies.

We are also planning to extend our molecular epidemiology studies by characterizing small non-cleaved lymphomas from other world regions. We have established collaborations in Egypt, India and South America for this purpose.

Pathogenetic Role of EBV in Small Non-cleaved Lymphomas

The presence of EBV DNA in most endemic tumors but only a small proportion of sporadic tumors has led to conceptual difficulties in reconciling a pathogenetic role for this virus in small non-cleaved lymphomas. We have examined EBV association in the context of breakpoint location on chromosome 8 and have noted an apparent relationship. Almost all far 5' breakpoints are associated with EBV (22 of 23), including all endemic tumors examined and 3 of 4 sporadic tumors. In sporadic tumors, immediate 5' breakpoints are rarely associated with the presence of EBV (1 of 15), although intron breakpoints are divided equally among EBV positive and negative tumors. This has suggested to us that EBV may provide an essential pathogenetic element when the breakpoint is in some locations, but other breakpoint locations, possibly combined with other, so-far unrecognized factors, may provide all elements required for neoplastic behavior. At present we are attempting to better define the relationship between breakpoint locations and EBV, and also to study the potential mechanisms whereby EBV could provide a necessary pathogenetic element. One possible mechanisms is that latently expressed EBV genes influence c-myc expression directly, i.e via the regulatory elements of c-myc. This is a likely possibility, since several latently expressed EBV genes are transactivators- i.e influence the expression of other EBV genes. Indeed, the mechanism whereby EBV effects B cell transformation is likely to involve the transactivation of cellular genes - possibly even c-myc. Thus, sometimes structural changes in the c-myc gene may be sufficient in themselves to effect deregulation and neoplasia, but in other cases an effect of EBV on one or more of the c-myc regulatory elements may be essential.

To examine this issue further, we have made a construct containing the entire presumptive c-myc regulatory region and attached it to a reporter gene (chloramphenicol acetyl transferase, CAT). We should, by transfecting this construct into EBV positive and negative cell lines, be able to determine, by the level of CAT activity, whether EBV genes directly influence c-myc expression. If this experiment yields positive results it should prove possible to identify the relevant EBV gene and the c-myc sequences it acts upon. In addition to this experiment, we are also examining the influence of EBV genes on the expression of the separated 1st exon of c-myc, again, containing most of the regulatory elements, by infecting EBV negative cell lines which have intron breaks, and in which, therefore, the regulatory region of c-myc, along with the first exon, remains on chromosome 8 when the protein coding region is translocated to chromosome 14.

Immunoglobulin Gene Expression in Small Non-cleaved Lymphomas.

We have continued our investigations into the expression of immunoglobulin genes in Burkitt's lymphoma, and now feel confident that not only are both kappa and lambda genes expressed in some cloned cell lines, but that hybrid immunoglobulin molecules containing both light chains are produced. This appears to be due to transcription from both rearranged and unrearranged light chain genes. In one set of cloned cell lines from the same parent, it appears that one of the clones contains a different lambda gene polymorphism from the others. Such a finding is unprecedented, and its significance is unknown. Identity of all other known genetic rearrangements in these cell clones has been confirmed. We hope that our studies of immunoglobulin gene rearrangements and expression in Burkitt's lymphoma will aid in the identification of the timing of the translocation in relationship to the physiological rearrangements occurring during B-cell differentiation. Our current interpretation of available data points toward the translocations occurring in a cell already containing a functionally rearranged mu gene. If this is so, the possibility that the translocation represents a mistake in VDJ joining - an hypothesis favored by some - is effectively excluded.

Antisense Regulation of c-myc Translation in Small Non-cleaved Lymphomas

We have pursued our objective of attempting to demonstrate that knowledge of the molecular abnormalities of a tumor may lead to novel treatment approaches by exploring the possibility of developing a means of specifically inhibiting the translocated c-myc gene in Burkitt's lymphoma while not affecting the c-myc gene of normal cells. We have chosen to use an antisense oligomer directed towards 1st intron c-myc sequences, since these are not present in normal c-myc transcripts, but are present in Burkitt's lymphomas which have 1st intron breakpoints. With this oligomer we have been able to inhibit proliferation completely in a cell line containing such an intron breakpoint. Other oligomers, such as the equivalent sense sequence, have no effect. We have also shown a reduction in c-myc protein in this cell line when treated with the anti-intron antisense oligomer. Additional confirmatory studies are in progress.

The ability to prevent translation of c-myc in Burkitt's lymphoma cell lines should also be of great value in exploring the functions of this gene. Since at present we have affected oligomer entry by simple diffusion, we are currently exploring the possibility of stably transfecting cell lines with a plasmid construct containing the same antisense sequences under the regulation of an inducible promoter. If this is successful, many experiments requiring large numbers of cells - presently limited by the very small quantities of oligomer that can be synthesized - should become feasible.

Experimental Use of a Small Non-cleaved Lymphoma Xenograft model

We have developed a xenograft model of Burkitt's lymphoma which we plan to make use of in many different ways. At present our efforts are directed towards understanding the differences in oncogenic potential in the nude mouse, which we have observed in our cell lines, including cloned lines from the same parent. The lines differ both with regard to the frequency of successful "takes", and also with regard to the rate of growth of individual tumors in different mice.

We are studying a variety of factors of potential importance in these regards, including the level of expression of various relevant genes, such as c-myc, and the level of surface expression of leukocyte adhesion molecules and receptors for growth factors such as Il-6 and transferrin.

This model should also be invaluable for the exploration of novel treatment approaches, possibly including c-myc anti-intron antisense molecules.

Treatment of Recurrent Sarcomas with Ifosfamide/VP16/Mesna

Protocol 85-C-154 has so far accrued 147 patients with recurrent sarcomas. The overall response rate to chemotherapy alone is 43% (42 partial responses and 8 complete responses among 115 patients evaluable for response to chemotherapy alone). This is almost double the response rate seen with ifosfamide as a single agent, particularly in the categories of Ewing's sarcoma and rhabdomyosarcoma. These results have influenced the design of primary therapy for sarcomas in both this and other institutions - insofar that these drugs have been incorporated into primary protocols.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 00650-33 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Service Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Eli Glatstein	Branch Chief	ROB, NCI
Others:	J. Bader	Chief, Clinical Radiotherapy Section	ROB, NCI
	T. DeLaney	Senior Investigator	ROB, NCI
	A. Raubitschek	Senior Investigator	ROB, NCI
	B. Kelly	On-Site Coordinator	ROB, NCI
	J. Grieg	Chief Technologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	4	6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to provide expert radiotherapy, consultation, and treatment for patients of the Clinical Center, including patients admitted to services other than the ROB. Support is given to the Medicine Branch, Surgery Branch, Pediatric Branch, NCI/Navy Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and others.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description

Professional Personnel Engaged on the Project:

J. Rowland	Nurse Specialist	CNS, CC
R. Smith	Clinical Nurse	CNS, CC
L. Walton	Clinical Nurse	CNS, CC
L. Dachowski	Clinical Nurse	CNS, CC

Methods Employed

Formal and informal consultation with referring physicians and application of radiotherapy where appropriate with x-rays and electrons in accordance with standard radiotherapy practice, as well as modified programs when necessitated by concomitant adjuvant therapies.

Major Findings

Just under 700 patients were seen in formal consultation this year. In addition, between 400 and 500 telephone conversations provided ad hoc advice on treatment for a variety of problems and general information, including nursing management and follow-up for radiation therapy related problems. Approximately three visits per month from nursing staff to observe delivery of radiation therapy and simulation process. Approximately 450 patients will be treated this fiscal year with most of these being protocol patients in the Radiation Oncology Branch, or on collaborative studies.

Proposed Course

To continue.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06310-09 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Surgery Versus Radiation Therapy in Treatment of Primary Breast Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J. L. Bader Chief, Clinical Radiotherapy Section ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Clinical Radiotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6	3	3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to determine whether a breast-conserving treatment program of limited surgery and definitive radiation offers equivalent local control and survival to mastectomy in patients with early stage breast cancer. After work-up confirms localized disease, patients are randomly assigned to either primary surgery or primary irradiation. Patients treated with mastectomy are offered breast reconstruction. All patients undergo complete axillary node removal; those patients with pathologically positive lymph nodes receive chemotherapy.

Project Description

Professional Personnel Engaged on the Project:

H. McDonald	Senior Surgeon	SB, NCI
D. Danforth	Senior Investigator	SB, NCI
M. Lippman	Head, Med. Brst. Cancer Sect.	MB, NCI
W. Schain	Clinical Care Consultant	Rehab. Med., CC
N. L. Gerber	Chief, Rehab. Medicine	Rehab. Med., CC
T. d'Angelo	Cancer Nursing Specialist	CNS, CC
M. Lampert	Physical Therapist	Rehab. Med., CC
S. Swain	Senior Staff Fellow	MB, NCI
L. Campbell	Staff Nurse	ROB, NCI

Objectives: If survival and recurrence data obtained with treatment that preserves a cosmetically acceptable breast are comparable to those obtained with radical surgical procedures, such treatment will probably be more acceptable to most women with localized breast cancer. Availability of an effective alternative to mastectomy may encourage women to seek medical attention with earlier, hence more curable, cancers. The cosmetic and functional results of local treatment will be carefully evaluated. The psychological, sexual and sociological impact of mastectomy vs. lumpectomy and radiation will be noted. Ability to combine aggressive cheomtherapy with either local treatment in node positive patients will also be assessed.

Methods Employed

Patients with stage T1-T2, N0-N1, M0 primary untreated breast cancer are candidates for the study. They will be randomized to receive either lumpectomy, axillary dissection and radiation therapy or total mastectomy with axillary node dissection. Patients receiving mastectomy will be offered breast reconstruction. Patients with pathologically positive lymph nodes will receive chemotherapy.

Major Findings

This study has been active for 9 years. Currently, 251 patients have been entered, of whom 126 have randomized to mastectomy, and 125 to radiation. Median follow-up is 54 months. No differences have been seen as yet between the two arms in terms of overall survival (~88% at 60 months). There have been 13 local/regional recurrences in the radiation arm. (Nine/twelve in-breast-only failures were salvaged by mastectomy.) Eight local/regional recurrences have occurred on the mastectomy arm.

Significance to Biomedical Research and the Program of the Institute

The study is intended to determine whether breast conserving treatment (lumpectomy and radiation therapy) is equivalent to radical surgery as treatment for early stage breast cancer. If this is the case, this treatment option should be much more acceptable to the majority of women. It is conceivable that the availability of such non-mutilizing treatment would encourage women to seek medical attention sooner, and therefore present with more curable disease.

Proposed Course

The study is ongoing, but will probably close within this calendar year.

Publications

None in 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM 06320-09 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Response of Mammalian Cells to Chemotherapy Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Cook	Associate	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Experimental Phototherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	4	2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Several chemotherapy agents with proven utility such as anthracyclines, bleomycins, alkylators, neocarcinostatin, nobel metal derivatives, VP-16, and hypoxic radiosensitizers are being studied. The detoxification mechanisms, modification of cellular response by altered intracellular redox status and oxygen metabolism in sensitive and resistant cells are of interest to the area of cancer treatment and directly related to our studies. Deleterious species produced by the antineoplastic drugs and cellular response to these species, as well as sulfhydryl containing compounds as they relate to metabolism, activation, and detoxification of antineoplastics. It has been demonstrated that depletion of glutathione levels either by directly conjugating or inhibition of de novo synthesis results in sensitization of cells by adriamycin, bleomycin, cisplatin, VP-16, alkylators, and hypoxic radiosensitizers. Alternatively, increasing glutathione levels by providing direct precursors results in protection of cells from the above reagents. Rescue of cells after treatment by supplying glutathione directly by modifying the molecule such that it becomes membrane permeable is being studied. Timing of delivery of the rescue agents, how the rescue agents interact with other biochemical pathways, cellular clearance and in vivo clearance are being investigated.

Project Description

Objective: The objective of this project is to determine the importance of biochemical modulation of selected cellular redox compounds upon chemotherapeutic drug cytotoxicity.

Methods Employed

In vitro cell culture and in vivo murine tumor models will be exposed to the various reagents mentioned above and assayed by conventional clonogenic assay, dye markers, tumor dose response, and survival advantage. In the in vivo studies, both thymic and athymic mouse are available to investigate murine and human tumor response. Standard biochemical enzyme assays, synthetic organic chemistry techniques, high performance liquid chromatography, and molecular biology techniques will and are being used.

Major Findings

Cell killing is enhanced for adriamycin, cis platin, bleomycin, VP-16, alkylating agents and hypoxic radiation sensitizers when glutathione levels are lessened in cells. Likewise, there is greater marrow effect when glutathione is modulated downward by several methods. Further, there is tissue differences as to response to depletion. Bone marrow may ultimately be more resistant to glutathione depletion than quickly growing tumors. Protection is provided by timing the glutathione depletion in such a fashion that both glutathione and glutathione-S-transferase levels rebound after depletion. Transport of rescue agents in vitro and in a murine in vivo system appears to be subject to intracellular levels of glutathione when metabolic precursors of glutathione are used; however, this may not be the case when analogues of glutathione are used.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding of drug-induced resistance and provide potential means of overcoming such resistant clones. Likewise, work is accumulating that may allow for differentiating normal tissue and tumor response to antineoplastic drugs by manipulating, in part, the redox status of cells.

Proposed course

To contine to explore the best means of modifying chemotherapy response by manipulation of redox cycles.

Publication

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06321-09 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Radiosensitization and Chemosensitization of Aerated and Hypoxic Mammalian Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	J. A. Cook	Staff Fellow	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A major portion of this study has dealt with the importance of cellular redox systems such as glutathione (GSH) and related enzymes to the cell's defense against ionizing radiation and chemotherapy drugs. Recent studies have clearly shown that inherent GSH levels do not significantly contribute to the radiation response. This statement is substantiated by our work using drugs that result in GSH depletion to <5% of control or elevation to 2 fold. Under these conditions, radiosensitivity was affected modestly (~10%). Recently using a mutant CHO cell line, radiosensitivity was found to be identical to the parental cell line, despite the fact that the mutant cell line had GSH levels 2 fold higher. Incidentally, this mutant cell line was markedly resistant to a wide variety of chemotherapy drugs. Thus, a link with chemoresistance and radiosensitivity was not found.

GSH modulation was found to significantly alter the chemotherapy response particularly for drugs such as melphalan and cisplatin. Particular attention was directed toward the development of an automated drug sensitivity (MTT assay) to screen a large panel (30 cell lines) of human lung cancer cell lines to determine if a relationship between chemosensitivity and GSH levels exists. Basically, the results showed that cell lines (small cell) low in GSH were more sensitive to cisplatin, melphalan, and adriamycin than cell lines 2-3 fold higher in GSH (non-small cell). The patterns of chemosensitivity observed correlated with what is seen clinically. These studies warrant further investigation as to the possibility of using GSH levels as a predictor of tumor response to chemotherapy drugs.

Project Description

Objective: The objective of the proposed project is to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death and to study the inter-relationships of factors which influence radiosensitivity and chemosensitivity, with an emphasis on their implications for the clinic.

Methods Employed

In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to radiation or selected chemotherapy drugs, either under aerated or hypoxic conditions. Oxygen enhancement ratios (OER) will be determined. Cellular GSH will be measured by spectrophotometric methods and cellular levels altered by drugs that specifically modulate the GSH cycle.

Major Findings

No relationship was found between chemoresistance and radiation sensitivity. GSH levels were found to significantly influence the response of cells to chemotherapy drugs. GSH is currently being studied and considered as a predictor of tumor cell response to chemotherapy drugs.

Significance to Biomedical Research and the Program of the Institute

These results show that modulation of tumor cell GSH can influence the cellular response to chemotherapy drugs. Agents such as buthionine sulfoximine (BSO) which inhibits GSH synthesis are being considered for chemotherapy clinical trials. Depletion of GSH by BSO could improve tumor treatment for selected chemotherapy drugs provided normal tissue toxicity is not also increased. More information is needed as to GSH levels in human tumors and normal tissues.

Proposed Course

More studies will be conducted at the cellular level on a more efficient means of GSH modulation. A major effort will be directed toward the measurement of GSH (and related enzymes) in human tumor and normal tissue.

Publications

1. Kurtzman S, Russo A, Mitchell JB, DeGraff W, Sindelar WF, Brechbiel MW, Gansow OA, Friedman AM, Hines JJ, Atcher RW. ^{212}Bismuth linked to an anti-pancreatic carcinoma antibody: A model for alpha particle emitting radioimmuno therapy, JNCI 1988;80: 449-452.

2. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium based semi-automated colorimetric assay: I Assessment of chemosensitivity testing, Cancer Res 1987;47: 936-942.

3. Mitchell JB, Russo A. The role of glutathione in radiation and drug induced cytotoxicity, Brit J Cancer 1987;55: 96-104.

4. Mitchell JB, Gamson J, Russo A, Friedman N, DeGraff W, Carmichael J, Glatstein E. Chinese hamster pleiotropic multidrug-resistant cells are not radioresistant, NCI Monographs 1988;6: 187-191.

5. Mitchell JB, Biaglow JE, Russo A. Role of glutathione and other endogenous thiols in radiation protection, Pharmac Ther, (in press).

6. Mitchell JB, Russo A, Carmichael J, Glatstein E. Glutathione as a predictor of tumor response. In: Prediction of tumor treatment response. New York: Pergamon Press, Inc., (in press).

7. Carmichael J, Mitchell JB, DeGraff WG, Gamson J, Gazdar AF, Johnson BE, Glatstein E, Minna JD. Chemosensitivity testing of human lung cancer cell lines using the MTT assay, Br J Cancer, (in press).

8. Carmichael J, Park JG, DeGraff WG, Gamson J, Gazdar AF, Mitchell JB. Radiation sensitivity and study of glutathione and related enzymes in human colorectal cancer cell lines, Eur J Cancer, (in press).

9. Mitchell JB. Glutathione modulation and cancer treatment. ISI Atlas of Science, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06329-08 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Radiation Physics Service

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech	ROB, NCI
	B. Arora Chin	Radiation Physicist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH Radiation Oncology Branch

SECTION Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION NCI,NIH,Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This section continues to provide expert physical and technological support for radiation treatment. This support consists of routine calibration and quality assurance of all radiation equipment and includes special dosimetry studies, computer-assisted treatment planning, and the design and development of special equipment tailored to special clinical needs. Regular checking of dosimetric and technical set-up aspects of radiation treatment will continue.

1. The improvement of the quality assurance (QA) program for the three Varian accelerators (Clinacs 4, 18, and 20) and the Scanditronix Microtron M22 is an ongoing effort. A new quality assurance detector using five ionization chambers has been integrated into the QA program. This device consolidates output, energy and symmetry checks and will be useful for electrons as well as photons.

2. Adaptation of the radiation equipment and special supporting equipment for patient treatment and its implemention is a continuing effort, continually adjusted also to the needs of the ongoing and new clinical research programs.

3. The Microtron is still under repair and acceptance testing will shortly start (probably around July 15).

4. The computer programs for clinical radiation treatment planning are currently being adapted for implementation on a Macintosh II system. This innovation promises portability, and easy implementation by and for other institutions. Support of intracranial implants continues to be of particular interest in this regard.

5. Extension of the clinical usefulness of the VAX-750 Computer System is also continuing. A MicroVAX system and several Macintosh systems have been added to improve the flexibility and to provide better support for ongoing research.

6. Supporting patient treatment and evaluation of clinical research.

Project Description

Personnel:

E. Lamoreaux	Computer Specialist	ROB, NCI
Huchen Xie	Computer Specialist	ROB, NCI
Junwen Chen	Radiation Physicist	ROB, NCI
P. Foley	Electronics Specialist	ROB, NCI

Objectives: To ensure highly flexible and quality physics support for radiotherapy.

Methods Employed

The locally developed highly efficient system for daily and periodic quality assurance is continually used for monitoring the performance of three linear accelerators, the Microtron, the simulator, and the CT scanner. Special mechanical supports and measuring devices are used to quantify the position of patients and to improve the reproducibility of daily patient set-ups. The data acquisition for treatment planning have been simplified and improved. Considerable efforts have been invested in the dosimetry of intraoperative, total-body, and total-skin radiotherapy. The restoration of the Microtron is nearing completion. In the meantime, the intraoperative work is continuing on the Clinac-20.

The Section continues to provide non-routine in vivo patient dosimetry by means of thermoluminescent dosimeters and diodes. Such ad hoc measurements are usually concerned with doses to sensitive organs, and are sometimes crucial to the continuation of a treatment technique.

Major Findings

Beam monitoring locally developed and other quality assurance support jigs enable daily monitoring of output, beam flatness, symmetry, and alignment of light field and x-ray fields for all three linear accelerators. The method allows simple documentation of performance. Our system continues to impress visitors. The dosimetry of photon beam total-body irradiation, as well as that of total-skin electron beam irradiation for mycosis fungoides, requires further attention especially in improving the treatment of hands and feet.

The most important contribution in computer-assisted treatment planning is the availability of routine interactive optimization and routine multi-slice imaging of dose distributions superimposed on CT scans. An important improvement is the capability to image the effects of irregular shielding blocks which is of essential interest in the treatment of soft-tissue sarcomas and cancers of the esophagus.

The use of locally designed and developed equipment and methodology continues to be a major factor in quality control of equipment, methodology and treatment documentation. This is especially important in view of the generally highly complex clinical studies in this Branch. Reliability of treatment delivery is being improved by implementation of a computer controlled hand-held bar code reader system, developed in-house by Robert Miller.

Significance to Biomedical Research and the Program of the Institute

The improvements in quality assurance, patient positioning, and treatment planning are essential as a basis for optimal patient treatment and for meaningful evaluation of treatment protocol studies. The CT scanner is now the principal source of patient data for treatment planning.

Proposed Course

1. Continuation of adaptation of the computer programs to the new radiation machines, with emphasis on an updated system based on the Macintosh II.
2. Special attention to the quality assurance aspects of the Microtron, currently under re-installation.
3. Integration of alternative imaging systems such as MRI and PET into the updated treatment planning system.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06330-08 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radiation Field Modeling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	Junwen Chen	Radiation Physicist	ROB, NCI
	Huchen Xie	Computer Specialist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is ongoing research and development. The capability to calculate the distribution of absorbed dose produced by photon beams and electron beams of the most general characteristics is fundamental to radiotherapy. The radiation field model has been described before. It takes as a basis the empirical distributions along three mutually perpendicular reference lines in a "master field." This concept is applied to the beam-modifying devices as well. One virtue of this approach is that it requires few experimental data and thus can be implemented very easily.

A completely new approach to electron field modeling has been developed and published in 1987. Its application to unit density media has been implemented. Extension to inhomogeneous media is underway. Of special interest are the implications for easy handling of different electron energies and field shapes in combination with photon fields.

Project Description

Objectives: To extend unified calculative models for the description of absorbed dose produced by beams of ionizing radiation, including photon beams as well as electron beams, as a basis for computer-assisted treatment planning, with special attention to high energy x-ray and electrons.

Methods Employed

Special attention will be paid to verification of the model for the revitalized Microtron, to the 6 MV and 21 MV x-ray beams as well as the electron beams. For this purpose, the newly updated Therados RFA-7 radiation field scanner is going to be of vital importance.

Major Findings

In x-ray dose field modeling, the description of electron transport correction has proved to be highly significant especially in high energy x-ray treatment with small fields in the thorax. Preliminary results for electron beams are most promising. The new electron beam model is both simple and more accurate than any other current approach. All of these results are being incorporated in a new clinical treatment planning system built around a Macintosh II computer.

Significance to Biomedical Research and the Program of the Institute

The range of validity of the dose field model determines the potential range of applicability of the clinical treatment planning program. In turn, the latter determines the degree of refinement in radiation treatment that can be scientifically documented. Current development could also become attractive for dissemination into the radiotherapy community, and improve the exchangeability of treatment documentation in clinical trials.

Proposed Course

This project to be continued,with the emphasis of inhomogeneities in photon and electron beams. In regard to electron beams, the influence of oblique incidence, non-standard distances between electron applicators, and patient surface need further attention, especially in view of moving electron beam. We will continue working with contracted outside experts for efficient development of specialized software, under our close control. Newly established interaction, within the NIH, with groups working on similar projects are already proving mutually productive and are to be continued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06331-08 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Computer-Assisted 3-D Radiation Treatment Planning

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	Huchen Xie	Computer Specialist	ROB, NCI
	Kathy Yeakel-Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4	1	3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this continuing project is the development and clinical implementation of a generalized system for external beam treatment planning. It will enable the optimum utilization of existing treatment facilities, using state of the art computer technology. The system is based on a generalized 3-D dose field model which covers photon and electrons, as well as neutron beams.

Work on the implementation of the Microtron with its 2 photon energies and 9 electron energies and some unusual technical options is currently on hold due to restoration and updating of the installation.
Work on implementation of a new locally developed electron beam model of great simplicity and accuracy is continuing.

The capabilities of the programs for interstitial treatment planning are of vital importance to the brain implant study.

Significance to Biomedical Research and the Program of the Institute

The convenient interactive manipulation of the key beam parameters in combination with fast response is highly valuable in the complicated dosimetry problems encountered in special protocol studies. The facility is also highly effective in the Resident's Training Program. Especially versatile 3-D imaging currently being implemented will greatly improve knowledge of dose distribution vs. anatomy.

The Macintosh II system will prove to be highly versatile and portable, thus being attractive to a broad community.

Proposed Course

1. Further development of the especially new imaging techniques particularly based on the new, highly versatile and inexpensive Macintosh II system.

2. Extension of the new locally developed electron beam model to cover inhomogeneity.

3. Establishment of a "Slave Monitor System" to enable the display and limited modification of treatment plans during the daily patient conferences; this is still being delayed by budgeting problems.

4. Extension of the capabilities to compute and display dose distributions in sagittal, coronal and Beam's Eye View sections of the patient on an interactive basis.

5. Development and implementation of digital x-ray imaging in conjunction with computerized treatment planning; this project is now nearing the clinical test phase.

6. Development of digital x-ray imaging in the realm of comparison of simulator (set-up) films and treatment machine port films. This, also, is now well underway.

Project Description

Personnel:

E. Lamoreaux	Computer Specialist	ROB, NCI
K. Yeakel-Orr	Dosimetrist	ROB, NCI
Huchen Xie	Computer Specialist	ROB, NCI

Objective: To develop and implement a generalized system for computer-assisted radiation treatment simulation.

Methods Employed

The dose field model for external beam dosimetry. The facility enabling the computation of display of dose distributions in planes perpendicular to the respective beam axes is being extended.

Currently, work is well underway to implement a suitably adjusted version of the various programs on a Macintosh II based system.

Major Findings

The Radiation Oncology Branch is continually engaged in innovative clinical studies. Detailed and accurate treatment planning is of essential concern, more so than in other oncology institutes. The quality, versatility and flexibility of our treatment planning system has proven to be indispensible to this research.

The system, although continuing to be further expanded, is in continuous routine use for clinical treatment planning. In comparison to other systems, operational as well as under development, it offers very high quality, high speed computation and display of complete dose distributions in multiple slices, superimposed on CT images, including rotation. The facility has a major impact on the conceptual understanding of the spatial aspect of radiation treatment dosimetry, as well as its application in clinical research programs.

Publications

1. van de Geijn J, Chin B, Pochobradsky J, Creecy RH, Miller RW. A new model for clinical electron beam dosimetry. Medical Physics 1987;14:577-584.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06351-06 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Response of Mammalian Cells to Halogenated Pyrimidines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.B. Mitchell	Senior Investigator	ROB, NCI
	J.A. Cook	Staff Fellow	ROB, NCI
Others:	A. Russo	Senior Investigator	ROB, NCI
	W. DeGraff	Biologist	ROB, NCI
	J. Gamson	Biologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

When certain halogenated pyrimidines such as bromodeoxyuridine (BrdUrd) and iododeoxyuridine (IdUrd) are incorporated into cellular DNA, cells become more sensitive to ionizing radiation and chemotherapy drugs. This observation has led to several clinical studies over the years and recently at the NCI to evaluate whether selective sensitization of tumors could be achieved by IdUrd infusion followed by radiation. An important question arises in these studies regarding the extent to which the drug is incorporated into cells. Work continues to develop techniques to quantify IdUrd incorporation into tumor and normal tissues. The IdUrd monoclonal antibody has proven useful in flow cytometry studies to accurately predict the labeling index (proportion of cells in S phase) and the laboratory is currently involved (with the clinical IdUrd study) in assessing this potentially important clinical parameter. Future studies will involve image analysis of tumor sections to gain insight as to spatial labeling patterns within the tumor. Quantitation of IdUrd replacement in tumor cell DNA is ongoing; however, due to tumor cell heterogeneity precise quantitation of this variable is difficult. We have been working with model rodent tumor systems in an attempt to work out sorting parameters prior to evaluation of clinical specimens. Lastly, using a semi-automated cell culture assay (MTT) we have established that sensitizers such as IdUrd can be easily be identified using this assay. Such large scale screening should allow for evaluation of new halogenated pyrimidine sensitizers.

Project Description

Objectives: To quantitate the amount of IdUrd in tumor vs. normal tissue by flow cytometry, HPLC, and image analysis. With these techniques, optimal timing schedules of incorporation for maximum differential radiosensitization will be determined.

Methods Employed

A monoclonal antibody for IdUrd and HPLC assays will be used to quantitate incorporation of IdUrd in tissues. Standard cell survival techniques have been used. Image analysis will be preformed using a fluorescent microscope linked to laser excitation and computer image analysis systems. A semi-automated growth rate assay was used employing tetrazolium.

Major Findings

Positive identification of cells in tumor and normal tissue that had incorporated BrdUrd and IdUrd has been made using the monoclonal staining technique. These studies should provide a better understanding as to quantities of IdUrd required to radiosensitize cells from tumor and normal tissue in a clinical setting. IdUrd incorporation was found to provide little enhancement of low energy photons of the energy associated with the K edge of iodine.

Significance to Biomedical Research and the Program of the Institute

These studies should provide a better understanding as to quantities and timing of IdUrd required to radiosensitize cells from tumor and normal tissue in a clinical setting. This technique will enable rapid determination of tumor labeling index. This parameter may be useful in selecting appropriate treatment approaches for tumors dependent on their cell cycle characteristics.

Proposed Course

Continue work on cellular quantitations of IdUrd. Evaluate cell survival of other mammalian cells to halogenated purines and pyrimidines and work out timing of incorporation for maximum differential sensitization. We will also attempt to alter the timing of pyrimidine and radiation for maximal sensitization.

Publications

1. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium based semi-automated colorimetric assay: II Assessment of radiosensitivity, Cancer Res 1987;47: 943-6.

2. Miller RW, DeGraff W, Kinsella TJ, Mitchell JB. Evaluation of incorporated iododeoxyuridine cellular radiosensitization by photon activation therapy, Int J Radiat Oncol Biol Phys 1987;13: 1193-7.

3. Mitchell JB. Potential applicability of non-clonogenic measurements to clinical oncology, Radiation Res, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06352-06 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relaxation Agents for NMR Diagnostic Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	O. A. Gansow	Senior Investigator	ROB, NCI
Others:	M. W. Brechbiel	Chemist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nuclear Magnetic Resonance (NMR) imaging is a most powerful method for the non-invasive diagnosis of disease. A fundamental limitation of the technique derives from the fact that images are constructed from T1 relaxation time measurements of protons in the various biological "compartments". If T1 values for differing soft tissue types are similar, the type will not, in general, be resolvable in the images. A potential method for improving this situation is the development of relaxation agents which specifically alter T1 relaxation rates in tissues where they may be concentrated.

We have recently shown that metal chelates are useful as an NMR contrast agent in myelography and cisternography. We have prepared a new and superior contrast agent, Gadolinium (Gd)DOTA. This chelate has recently been shown to be more stable in vivo than Gd(DTPA).

This year, we report some new applications of contrast agents for in vivo MRI imaging.

Project Description

Professional Personnel Engaged on the Project:

J. Frank	Clinical Associate	DR, CC

Objectives: We proposed to construct paramagnetic molecules that localize in certain biological compartments in order to reduce T1 relaxation times of water in the area. We plan to attach paramagnetic metal chelates to proteins found to localize where desired in the body. The idea is that since paramagnetics alter local T1 values, by concentrating them in differing tissue types, we could induce resolution in NMR images. For example, paramagnetic labels attached to blood proteins, which circulate freely blood, thus allowing imaging of cardiac function and blood flow. A second example would be to label tumor associated monoclonal antibodies. In recent work done in this Section, it has been proven possible to localize radioisotopes attached to antibodies in tumors by using metal chelates.

Methods Employed

Bifunctional metal chelates capable of securely binding paramagnetic metals like iron, chromium or gadolinium have been prepared and attached to the proteins described above. The effect of these paramagnetic relaxation agents on T1 values have been measured by conventional inversion, recovery methods. The chelate, Gd (DOTA), is being tested as an MRI contrast agent (CA) by examination of relaxation times in aqueous media and in tissues excised from appropriate animals. *In vivo* studies of the MRI of CA enhanced tumors in mice are also in progress.

Major Findings

Studies have now shown that Gd(DTPA) may be attached to antibodies or albumin without affecting the biological properties of the proteins. Results of T1 studies show that many paramagnets must be attached to one protein to have an effect *in vivo*.

Use of Gd(DTPA) as an NMR contrast agent has demonstrated that MRI myelography and cisternography may be practically useful in the clinic.

We have prepared kilogram quantities of a new contrast agent Gd(DOTA) for use in humans.

In addition, considerable effort has been expended in devising methods for linkage of Gd(DOTA) to proteins to serve as a T1 relaxation agent. The most recent syntheses will now provide adequate quantities of metal chelate for trials in animals to proceed.

Publications:

1. Knop R, Frank J, Dwyer AJ, Girton M, Naegele M, Schrader M, Cobb J, Gansow OA, Magerstadt M, Brechbiel M, Baltzer L, Doppman JL. Gadolinium cryptelates as water soluble MRI contrast agents, J Computer Assist Tomography 1987;11:35-42.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06353-06 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Metal Chelate Conjugated Monoclonal Antibodies for Tumor Dx & Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	O. A. Gansow	Senior Investigator	ROB, NCI
Others:	S.M. Mirzadeh	Expert	ROB, NCI
	M. Brechbiel	Chemist	ROB, NCI
	T. McMurray	Senior Staff Fellow	ROB, NCI
	K. Kumar	Senior Staff Fellow	ROB, NCI

COOPERATING UNITS *(if any)*

Johns Hopkins Medical School, Baltimore, MD (M. Strand); Argonne National Laboratory, Argonne, IL (R. W. Atcher)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Tumor-associated monoclonal antibodies are potential therapeutic agents as selective carriers of cytotoxic agents to malignant cells. We are testing this hypothesis in several animal model systems: one is a tumor virus induced leukemia of mice; another is human tumor xenographs in nude athymic mice.

The various cytocidal agents being employed are radioisotopes. Their relative therapeutic efficacy when conjugated to antibodies is being assayed and compared to that of monoclonal antibodies alone. The isotopes now being used include the highly tumoricidal alpha emitting parent radioisotopes Pb-212 or Bi-212, as well as the beta particle emitter, Y-90. The syntheses of different chelates and radiochemical separations required for these objectives are being devised and reduced to clinical practice. Results from isotopic therapy are being compared with those obtained by use of antibody conjugated toxins or drugs with respect to tumor growth, regression, or cure.

These studies will provide for human medicine a basis for design of rational therapy of malignancies by selectively targeting cytocidal agents to tumors, as well as metastases.

New chelates for use in this project have been synthesized and used, and have thus far proven useful for radiobiology studies of cell killing with alpha particle labeled antibody and for imaging of tumors in mice. Studies of tumor imaging in patients has begun. Pre-clinical examination of Y-90 labeled antibodies for therapy are in progress.

Project Description

Professional Personnel Engaged on the Project:

D. Colcher	Senior Investigator	LCMB, NCI
T. Waldmann	Chief	MET, NCI
R. Kozak	Staff Fellow	MET, NCI

Objectives: The specific goal of these studies is to investigate *in vitro* and in animal tumor models the therapeutic efficacy of radionuclides attached to tumor-associated monoclonal antibodies. These studies encompass the synthesis of new bifunctional chelates designed for therapy employing a variety of radioisotopes and radiation types.

Methods Employed

Methods for covalently conjugating metal isotopes in bifunctional chelates to monoclonal antibodies are being devised and developed. The inorganic chemistry of new complexing agents for metal isotopes thought to be useful in tumor diagnosis or therapy is being explored. The objectives of the research must thereby of necessity include: (a) the synthesis and characterization of new bifunctional chelates and their metal complexes, both before and after protein conjugation; (b) the evaluation of currently available chelates for use as carriers of isotopes familiar in clinical environments (e.g., Tc-99M) and of less common, but potentially serviceable radionuclides (e.g., Ga-68, In-111, Pb-212, Bi-212, Y-90); (c) the development of chemical procedures (protocols) for routine and reproducible preparations of rigorously stable radiometal chelate conjugated monoclonal antibodies which retain their inherent biological specificity and activity; and (d) the use of animal models for investigating the stability *in vivo* of metal labeled antibodies.

Major Findings

Some of the problems which have been addressed include: (1) the incorporation of Bi-212 into chelates attached to antibody; and (2) the evaluation of three DTPA chelates and an EDTA chelate for use in antibody modification.

1. A new generator for the Pb-212, Bi-212 radionuclides is now being provided by Argonne National Laboratory, and is in use to label antibodies for radiobiology studies. Studies completed show specific killing of targeted cells in an animal model.

2. Four chelates have been used for antibody labeling; the DTPA dianhydride, the DTPA isobutycarboxycarbonic anhydride, the paraisothiocyanatobenzyl derivatives of DTPA and EDTA. Methods for labeling antibody with In-111 and Y-90 have been developed. It was found that the benzyl DTPA chelate

gave superior tumor images when used to label antibody BF2.3 with radioindium. Of these chelates, only the benzyl DTPA is stable in vivo.

3. A study of the relative cytotoxicity of x-particle linked antibody vs. conventional gamma radiation (CS-137 beam) was performed. The radioimmunotherapy was shown to be superior.

4. New chelates for Pb-212, Bi-212, and Y-90 have been synthesized.

Significance to Biomedical Research and the Program of the Institute

The ability to attach metals to antibodies is significant for several reasons. It enables one to diagnose and detect cancer using radioactive metals in nuclear medicine tests, or using paramagnetic metals to enhance nuclear magnetic resonance images. The ability to attach particle emitters to antibodies opens up site specific therapy using a variety of radioactive metals which can be selected to maximize cell killing while sparing normal tissue. Finally, it appears that the bifunctional chelates currently being investigated have little effect on the viability and specificity of the antibodies, thus preserving their function.

Proposed Course

The effects of the number of chelates attached to the antibodies and the other conditions of preparation on the activity of the antibodies both in vitro and in vivo will be examined. Next, the conditions under which the metals are incorporated into the chelates will be examined for their effects on biological activity both in vitro and in vivo.

The radiobiology studies now underway will be expanded to test a number of human cell lines. Those cell lines adequately treatable will be proposed for study in animal models.

Protocols for diagnosing colon cancer in humans have been implemented in the clinic.

Protocols for treatment of T-cell leukemia and lymphoma are in place. Methods for obtaining pharmaceutical grade labeled antibody are developed.

Publications

1. Black CDV, Atcher RW, Barbet J, Brechbiel MW, Holton OD, Hires JJ, Gansow OA, and Weinstein JN. Selective ablation of B lymphocytes in vivo by an alpha emitter, 212-bismuth, chelated to a monoclonal antibody, Antibody, Immunoconjugates and Radiopharmaceuticals 1988;1:43-53.

2. Gansow OA, Brechbiel MW, Mirzadeh S, Colcher D, and Rosselli M. Chelates and antibodies: Correct methods and new directions. In: Goldenberg D, ed. Monoclonal antibodies for tumor diagnosis and therapy, in press.

3. Kurtzman SH, Russo A, Mitchell JB, DeGraff W, Sindelar WF, Brechbiel MW, Gansow OA, Friedman AM, Hines JJ, Gamson J, Atcher RW. 212-Bismuth linked to an antipancreatic carcinoma antibody: Model for alpha-particle-emitter radioimmunotherapy, J Nat Cancer Institute 1988;80:449-52.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06354-06 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Iron-57 & H-2 Nuclear Magnetic Resonance: New Tools

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: O. A. Gansow	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

University of Goteborg, Goteborg, Sweden (Dr. L. Baltzer)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are developing iron-57 and deuterium Nuclear Magnetic Resonance (NMR) as an experimental method for use in the Biomedical Sciences. Many compounds found in the blood contain iron cores. Among these are the cytochromes, hemoglobin, or ferridoxin. Few chemical methods may be used to directly study the central metal atom environments of these proteins. Iron-57 NMR is being developed for that purpose.

Some time ago, we reported the utility of iron-57 NMR for study of relaxation times and chemical shifts of some iron compounds related to biological entities. We determined thus the basic parameters needed for further study of iron NMR.

Our current efforts to develop the method have involved synthesis of series of weakly magnetic iron-57 enriched compounds. Efforts to observe the metal resonance are underway.

Project Description

Professional Personnel Engaged on the Project:

E. Becker	Section Head	LCP, NIAMS

Objectives: We plan to measure the iron and deuterium NMR signals from a number of biological, inorganic, and organometallic compounds. The goal of the project is to define the experimental conditions and parameters necessary for the direct detection of iron and deuterium NMR in order to explore its utility for studies of biological processes.

To develop the method, a knowledge of two physical parameters of iron NMR signals must be obtained. They are chemical shift values and T1 relaxation times. By observing resonances of inorganic and organometallic model compounds, it is possible to define the chemical shift scale for the iron nucleus. These data serve to define the resolution of the method. Similarly, by measuring T1 relaxation times of these compounds, we will learn how to optimize chemical environments and experimental conditions required to detect iron resonance. With that information in hand, a rational selection of biological problems amenable to study by this method can be effective.

Methods Employed

A specially constructed NMR probe for observing iron NMR has been built and used as described in our recent publication. Enriched iron-57 proteins such as myoglobin have been synthesized. A new NMR probe for use with biological molecules has been constructed and tested successfully. Probes for H-2 NMR have also been employed to measure H-2 NMR of porphyrins.

Major Findings

We have accomplished the first direct detection of iron-57 NMR in biological molecules. We have undertaken the first systematic study of iron-57 chemical shift values. Initial results indicate a range of $>$5000 ppm. A study of iron-57 relaxation times showed the advantage of the experiment being done at high magnetic fields.

Synthesis of the iron (tspp) porphyrin enriched in iron-57 has been completed. The compound has been sent to the laboratory of Dr. Baltzer in Sweden for analysis on an especially designed probe for iron-57 NMR. In addition, a number of other weakly paramagnetic iron complexes have been selected for study and synthesized for this project.

Significance to Biomedical Research and the Program of the Institute

Our studies have shown that iron-57 and H-2 are new and valuable methods for study of biological compounds. We have shown that 57-Fe NMR of proteins can be observed. We have developed a simple method for measuring magnetic anisotrophies of the iron porphyrins. Most biomedically important iron compounds are at least weakly paramagnetic. Thus, our current efforts are directed towards learning how to study the iron-57 NMR of such species.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06356-05 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Treatment of Malignant Brain Tumors with Interstitial Radiotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	K. Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Therapy Section and Physics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Median survival of high-grade gliomas remains less than a year, despite multi-modality treatment. Cure is considered anecdotal. These tumors usually have extended beyond the limits of a complete surgical resection, and the dose of conventional external beam radiotherapy has been limited by surrounding normal brain tolerance. We believe that we can achieve a higher radiation dose to the tumor by placing radioactive seeds of Iodine-125 directly into the tumor bed, with a sharp fall-off of radiation to the surrounding normal brain. Hopefully this will achieve a much better therapeutic ratio, especially when delivered at low dose rates.

Project Description

Professional Personnel Engaged on Project:

D. C. Wright	Senior Investigator	SN, NINCDS

Objectives

To develop a technique of interstitial implantation of intracranial tumors; to determine the acute effects and complications of such treatment; to explore the efficacy of such therapy; and to develop patient selection guidelines for future applications.

Methods Employed

Patients with primarily untreated high-grade gliomas of less than 5 cm diameter receive approximately 4000 rads of external beam radiotherapy prior to implantation. Implants of similar tumors recurrent after prior standard treatment receive implant only. Using a Brown Robert Wells stereotactic frame and a customized template device, silastic catheters loaded with radioactive seeds of Iodine-125 are stereotactically positioned in the tumor. Catheters are then anchored to the dura, and the bone defect is closed. Catheters are left in place to deliver an appropriate radiation dose, and then are removed at the time of a second, minor surgical procedure.

Major Findings

Twenty-two patients have been enrolled in the protocol. Nine patients with recurrent tumors received implants. Six patients died in less than 1 year, while three are alive at follow-up times after implantation of 2-12 months. Thirteen patients with no previous treatment have been entered on the protocol. Six patients died less than 1 year after implantation; five lived for greater than 1 year, ranging from 14-45 months, but subsequently died. Two patients are still alive, 3 and 21 months after treatment.

The technique for the stereotactic placement of multiple catheters containing multiple radioactive sources has been developed. It can be used to implant a tumor in any cranial site, excluding the posterior fossa, which is simply inaccessible. It can be adapted to a variety of isotopes and a variety of tumor configurations.

Significance to Medical Research and the Program of the Institute

This study helps to provide information on dose and dose rate effects on both tumor and normal brain after interstitial implantation of radioactive sources.

Proposed Course

It is proposed to study a total of 30 patients. We wish to explore the development of advanced computer algorithms for radiation treatment planning and dose display. Depending on the results of treatment of the first 30 patients, a decision will be made about whether to enter into additional studies with interstitial implantation of brain tumors.

Publications

1. Rosenow UF, Findlay PA, Wright DC. The NCI: Atlas of dose distributions for regular 125-I brain implants, Radiother Oncol 1987;10:127-39.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06357-05 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Clinical Studies on Intraoperative Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Glatstein	Chief	ROB, NCI
Others:	W. Sindelar	Senior Investigator	SB, NCI

COOPERATING UNITS *(if any)*

Surgery Branch

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
10	100	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The Radiation Oncology Branch and Surgery Branches of the National Cancer Institute have been involved in prospectively randomized studies evaluating the potential role of intraoperative radiation therapy in several disease sites, including resectable and unresectable carcinomas of the pancreas, resectable carcinomas of the stomach, and retroperitoneal sarcomas. One hundred patients have been treated with experimental intraoperative radiation therapy, and randomized to either receive or not receive radiation therapy intraoperatively with large single doses of electrons. There is really no suggestion of improvement in survival, or in disease-free survival. There is some suggestion of an improvement of local control in the retroperitoneum itself; however, this is off-set by a high predilection for seeding of the abdominal cavity, either peritoneal carcinomatosis or sarcomatosis, thus neutralizing the potential benefit of intraoperative radiation. The trials on pancreatic carcinoma and retroperitoneal sarcomas have been closed. The gastric study is still open for patient accrual.

Project Description:

Professional Personnel Engaged on the Project:

W. Sindelar	Senior Investigator	SB, NCI
H. Pass	Senior Investigator	SB, NCI
R. Smith	Cancer Nursing Specialist	CNS, CC

Objectives: These are phase I and II studies assessing the role of intraoperative radiation therapy as an adjunct to surgical resection in various primary tumor sites, including pancreas, stomach, and retroperitoneum, where local failure following surgery alone is extremely high. Additional pilot studies are ongoing to determine the role of intraoperative radiation therapy with tumors with high-risk of local recurrence.

Methods Employed

Patients are considered for entry on the randomized studies with combined surgical resection and intraoperative therapy that have specific malignant lesions with the abdomen and retroperitoneum, and lack evidence of metastatic spread. In general, the control arm of these studies receives resection with post-operative conventional fractionated radiotherapy, and the experimental arm receives in addition, intraoperative radiation therapy, as well as misonidazole, a known radiosensitizer of hypoxic cells, a single injection of 3.5 gm/m^2. Patients are followed closely to assess local toxicity, and patterns of recurrence.

Major Findings

With over 100 patients having been randomized to receive intraoperative radiation therapy at the NCI, there is no trend to suggest an improvement in local control, disease-free survival, or overall survival. Local control can be made to look quite good, if one talks only about the retroperitoneum. However, the marked predilection for carcinomatosis or sarcomatosis of the peritoneal surface itself, negates this potential gain. Until this problem can be overcome, intraoperative radiation therapy will not be useful on a large scale. Potentially, intraperitoneal chemotherapy, pre-operative radiation therapy, or intraoperative photodynamic therapy might be useful in overcoming this problem.

Significance to Biomedical Research and the Program of the Institute

Intraoperative radiation therapy studies are the first prospective randomized trials looking at this method of delivering radiation therapy.

Proposed Course

With the renovations of the electronics of the Microtron, we hope to continue these pilot trials. However, until we are able to deal realistically with the problem of peritoneal seeding, this modality will probably not prove to be useful. If we can overcome the problem of peritoneal seeding, this may represent a useful advance in a number of abdominal neoplasms. Photodynamic approaches to prevent peritoneal seeding are presently in phase I studies.

Publications

1. Pass HI, Sindelar WF, Kinsella TJ et al. Delivery of intraoperative radiation therapy (IORT) after pneumonectomy: Experimental observations and early clinical results, Annals of Thoracic Surgery 1987;44:14-20.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06358-05 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radiolysis, Photolysis and Sonolysis of Cells and their Constituents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Riesz	Research Chemist	ROB, NCI
Others:	Murali Krishna Cherukuri	Visiting Associate	ROB, NCI
	T. Kondo	International Research Fellow	ROB, NCI
	A. E. Alegria	MARC Fellow	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Experimental Phototherapy

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The photochemistry of the antitumor drugs adriamycin, daunomycin, certain quinolinium salts, and gilvocarcin V was investigated in relation to the possible combination of chemotherapy with photodynamic therapy. By the use of electron spin resonance and spin trapping techniques, and of (17-0) enriched water and oxygen, the relative contributions of superoxide radicals, hydroxyl radicals, and singlet oxygen to the photochemistry of these antitumor drugs was elucidated. The chemical effects of ultrasound were studied in relation to hyperthermia (used in combination with radiation therapy) and the possible consequences of diagnostic and therapeutic applications. The effect of varying the temperature of cavitation bubbles in the presence of different rare gases on free radical formation and on the shearing stress induced by ultrasound on mammalian cells was measured. The hydroxyl radical yields were in the order xenon >krypton>argon>neon>helium in accord with the higher temperatures of the cavitation bubbles. However, mammalian cell lysis induced by shearing stress was the same for all rare gases and independent of their thermal conductivity and the temperature of the cavitation bubbles. The radicals generated in aqueous solutions of nucleic acid and protein components by ultrasound have been identified by spin trapping and compared with those formed by ionizing radiation and by UV-photolysis in the presence of hydrogen peroxide. The observed isotopic exchange reactions suggest the possible use of sonochemical methods for the synthesis of deuterated protein and nucleic acid constituents.

Project Description

Objectives: The effects of ionizing and ultraviolet radiation and of ultrasound on biological macromolecules and their constituents are being investigated. ionizing radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals, or by the "indirect effect" where radical species are hydrated electrons, hydrogen atoms and hydroxyl radicals.

In the chain of events that lead to loss of biological activity, free radicals play an important role. Chemical compounds have been discovered which significantly modify radiation effects. These include: (a) electron affinity sensitizers which act on hypoxic tumor cells; (b) halogenated pyrimidines which are incorporated into DNA; and (c) cancer chemotherapy agents of the intercalating or alkylating type which sensitize tumor and normal cells. Studies of the mechanism of action of radiosensitizers and radioprotectors are necessary to design improved combinations of chemotherapy and radiation therapy.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity in macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

In the last few years, it has become apparent that superoxide anion radicals and hydroxyl radicals are found in many biological systems in the absence of either ionizing radiation or UV-photolysis. Recent reports have indicated that radicals are produced in the presence of certain anti-cancer drugs such as Bleomycin and Adriamycin. The significance of radical reactions is therefore not confined to radiation biology. It has also been shown that damage to tissues following ischemia appears to occur during reperfusion with oxygenated blood. This damage is generally considered to be due to the excessive production of superoxide radicals and hydrogen peroxide. In support of this hypothesis, it has been shown that in several model systems superoxide dismutase, catalase or allopurinol (a xanthine oxidase inhibitor) protect ischemic tissue from oxidative damage during reperfusion.

Methods Employed

Nucleic acids, proteins and their constituents were gamma-irradiated either in the solid state or in aqueous solutions in a 800-curie Colbalt gamma-source. Electron spin resonance studies were carried out with a Varian E-9 Spectrometer connected to an IBM-XT computer. For photolysis studies at specific wavelengths, a 1000-watt high pressure Xenon arc source and monochromator were employed. For ultrasound exposures, aqueous solutions were insulated in a non-perturbing cylindrical cell with 1 mil mylar windows in an anachoic ultrasound exposure apparatus at 30 ± 0.5 degrees C. Specimens were exposed to either continuous wave or tone bursts of 1 MHz ultrasound to simulate both therapeutic and diagnostic exposure conditions. In the spin trapping method, the short-lived free radicals react with a diamagnetic scavenger (the spin trap) to produce longer-lived radicals (the spin adduct) which can be conveniently investigated by e.s.r. In our studies, 2-Methyl-2-Nitrosopropane and 5,5-Dimethyl-1-Pyroline-1-Oxide were employed as the spin traps.

Major Findings

I. Free Radical Formation and Cell Lysis Induced by Ultrasound in the Presence of Different Rare Gases (with T. Kondo, J. Gamson and J.B. Mitchell)
The effect of varying the temperature of cavitation bubbles on free radical formation and shearing stress induced by 50 kHz ultrasound was investigated. The hydroxyl radical yields were in the order Xe>Kr>Ar>Ne>He in accord with the higher temperatures of the cavitation bubbles. However, there was no difference among rare gases for lysis of CHO cells. These results suggest that free radical formation is directly related to the final temperature of collapsing cavitation bubbles, but shearing stress which causes cell lysis is not.

II. Effect of Shear Stress and Free Radicals Induced by Ultrasound on Erythrocytes (with H. Kon, Y. Fukushima, Laboratory of Chemical Physics, NIDDK, NIH) and T. Kondo)
The hemolysis of erythrocytes induced by ultrasound was studied in the presence of argon and nitrous oxide to distinguish between cavitation with and without free radical formation. Hemolysis, membrane fluidity, membrane permeability and membrane deformability were examined. Hemolysis induced by sonication is due to mechanical shearing stress arising from cavitation, and membrane integrity of the unlysed erythrocytes after sonication was the same as that of control cells without sonication. Nitrous oxide is useful for ultrasonically disrupting cells without accompanying free radical formation.

III. Sonochemistry of Aqueous Solutions of Nucleic Acid Constituents (with T. Kondo and Murali Krishna Cherukuri)
The sonolysis of aqueous solutions of various dihydro and substituted pyrimidines was investigated by electron spin resonance (ESR) and spin trapping with the non-volatile, water soluble, deuterated spin trap 3,5 - dibromonitrosobenzene sulfonate (DBNBS - d_2). Similar ESR spectra were obtained from the ultraviolet photolysis of aqueous solutions containing hydrogen peroxide and from gamma-radiolysis of nitrous oxide saturated solutions, although sonolysis of aqueous solutions leads to the formation of pyrimidine radicals by H atom, as well as OH radical addition to the 5,6 double bond of pyrimidines.

IV. Effect of Non-volatile Scavengers of Hydroxyl Radicals on Thymine Radical Formation Induced by Gamma-rays and Ultrasound (with T. Kondo and Murali Krishna Cherukuri)
In order to investigate the mechanism of sonolysis of nucleic acid constituents, the yield of thymine radicals generated by ultrasound in argon-saturated aqueous solutions was compared with that formed by gamma radiolysis in N_2O - saturated solutions in the presence of non-volatile scavengers, which cannot act in the gas phase of the cavitation bubbles. The results show that thymine radicals induced by ultrasound are produced in the bulk of the solution as well as in the interfacial region.

V. Thermal Decomposition of Alcohols in the Sonolysis of Alcohol - Water Mixtures. Evidence for Isotope Exchange Reactions (with Murali Krishna Cherukuri and T. Kondo)
The spin trap DBNBS has been used to monitor the yield of free radicals produced during sonolysis of water - alcohol mixtures. From methanol, $CH_3\cdot$ and $\cdot CH_2OH$ radicals were observed, as well as the isotopically mixed radicals $\cdot CH_2D$ and $\cdot CHD_2$ when $CH_3OD:D_2O$ mixtures were studied. The results clearly show that thermal

decomposition of methanol to methyl radicals occurs in the gas phase. Ethanol, 1-propanol, 2-propanol, and tert. butyl alcohol have also been studied. Our results suggest the possible use of ultrasonic methods for deuterium labeling or organic compounds.

VI. Photochemistry of Aqueous Adriamycin and Daunomycin. A Spin Trapping Study with 17-0 Enriched Oxygen and Water (with A.E. Alegria)
The photochemical mechanisms leading to the formation of the spin adduct DMPO-OH from 5,5 dimethyl - 1 - pyrroline - 1 - oxide (DMPO) were elucidated using $^{17}0$ labeled oxygen and water.

VII. Photochemistry of Aqueous Solutions of Quinolinium salts. A Spin Trapping Study using ($^{17}0$) Enriched Water and Oxygen (with O.Cox, J. Dumas, L. Rivera (Dept. of Chemistry, University of Puerto Rico, Humacao)
The photochemistry of aqueous solutions of the antitumor drug 3 - nitro - 7 - ethylbenzimidazolo (2,2-a), quinolinium chloride at 344 nm was studied. Only a water-derived DMPO-OH spin adduct was obtained. A photosolvated intermediate is postulated as the OH donating species.

VIII. An ESR Study of the Visible Light Photochemistry of Gilvocarcin V (with R.K. Elespuru (Lab. of Chemical and Physical Carcinogenesis, LBI - Basic Research Program, NCI, Frederick), Murali Krishna Cherukuri and A. Alegria)
The photochemistry of gilvocarcin V (GV), a planar, DNA-intercalating C - glycoside isolated as a natural product antibiotic was investigated. GV photochemistry proceeds by Type I (electron transfer) and Type II (singlet oxygen generation) pathways which can contribute to the reported CV phototoxicity in biological systems.

IX. Superoxide Reaction with Nitroxide Spin Adducts (with A. Samuni, Murali Krishna Cherukuri, E. Finkelstein and A. Russo)
The reaction of superoxide radical with persistent nitroxide spin adducts and with stable spin labels were studied by ESR.

Significance to Biomedical Research and the Program of the Institute

Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy; (2) carcinogenesis; (3) stability of the genetic pool; (4) the suppression of the immune mechanism; and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal cells, and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained.

Proposed Course

To continue studies on the effects of ionizing radiation on mammalian cells and macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents and the interaction of radiation and cancer chemotherapy agents will be investigated. New areas of interest include photosensitized cell killing by porphyrins and phthalocyanines in relation to photodynamic therapy and chemical and biological effects of ultrasound.

Publications

1. Murali Krishna C, Decarroz C, Wagner JR, Cadet J, Riesz P. Menadione - sensitized photooxidation of nucleic acid and protein constituents: An ESR and spin trapping study, Photochem Photobiol 1987;46:175-182.
2. Rosenthal I, Riesz, P. Electron spin resonance and spin-trapping studies of radiation damage in biologically significant molecules, Radiat Phys Chem (Int J Radiat Appl Instrum Part C) 1987; 30:381-7.
3. Sonoda M, Murali Krishna C, Riesz P. The role of singlet oxygen in the photohemolysis of red blood cells sensitized by phthalocyanine sulfonates, Photochem Photobiol 1987;46:625-631.
4. Riesz P, Murali Krishna C. Phthalocyanines and their sulfonated derivatives as photosensitizers in photodynamic therapy, Proc SPIE-Int Soc Optical engineering 1988;847:15-28.
5. Rosenthal I, Murali Krishna C, Yang GS, Kondo T, Riesz P. A new approach for EPR detection of hydroxyl radicals by reaction with sterically hindered cyclic amines and oxygen, FESB Letters 1987;222:75-8.
6. Murali Krishna C, Kondo T, Riesz P. Sonochemistry of aqueous solutions of amino acids and peptides: A spin trapping study, Radiat Phys Chem (Int J Radiat Appl Instrum Part C) 1988;32:121-8.
7. Kondo T, Murali Krishna C, Riesz P. Free radical generation by ultrasound in aqueous solutions of nucleic acid bases and nucleosides: An ESR and spin-trapping study, Int J Radiat Biol 1988;53:331-342.
8. Murali Krishna C, Lion Y, Kondo T, Riesz P. Thermal decomposition of methanol in the sonolysis of methanol - water mixtures: Spin trapping evidence for isotope exchange reactions, J Phys Chem 1987;91:5847-50.
9. Kondo T, Murali Krishna C, Riesz P. Sonochemistry of nucleic acid constituents in aqueous solution: A spin-trapping study. In: Simic M, Ward J, Taylor A, eds. Oxygen radicals in biology and medicine. New York: Plenum Press, 1988 (in press).
10. Kondo T, Murali Krishna C, Riesz P. Effect of non-volatile scavengers of hydroxyl radicals on thymine radical formation induced by gamma-rays and ultrasound, Int J Radiat Biol 1988 (in press).
11. Kondo T, Murali Krishna C, Riesz P. Sonolysis, radiolysis, and hydrogen peroxide photolysis of pyrimidine derivatives in aqueous solutions: A spin trapping study, Radiat Res 1988 (in press).

12. Alegria AE, Riesz P. Photochemistry of aqueous adriamycin and daunomycin. A spin trapping study with ^{17}O enriched oxygen and water, Photochem Photobiol 1988 (in press).

13. Alegria AE, Cox O, Dumas J, Rivers LA, Riesz P. Photochemistry of aqueous solutions of quinolinium salts: A spin strapping study using (^{17}O) enriched water and oxygen, Biochem Biophys Acta 1988 (in press).

14. Samuni A, Murali Krishna C, Riesz P, Finkelstein E, Russo A. Superoxide reaction with nitroxide spin-adducts, Free Radicals in Biol and Med 1988 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06359-05 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phase I/II Study IUdR Given Intravenously in Conjunction with FUdR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. J. Kinsella	Senior Investigator	ROB, NCI
	E. Glatstein	Chief	ROB, NCI
Others:	Jan Rowland	Nurse	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Radiation Oncology Branch has demonstrated that IUdR, given by continuous intravenous infusion for 24 hours a day for up to 14 days, can function as an effective radiosensitizer in high-grade primary brain tumors, and other poorly responsive tumors. The treatment protocol involves two separate two-week infusions of IUdR combined with twice daily radiation therapy. The purpose of this study was to combine IUdR with modest doses of FUdR in an attempt to increase the uptake of IUdR within tumor cells by means of blocking de novo thymidine synthesis. The reasoning was that IUdR competes with thymidine in dividing cells, and thus by blocking thymidine synthesis, IUdR can be expected to be more effective. Thirty-five patients were treated with a variety of diagnoses, but mostly sarcomas and gliomas. Escalating doses of FUdR were given, along with escalating doses of IUdR. There was a marked increase in toxicity, not only in terms of metabolic toxicity, but also abnormal liver function tests, and severe mucositis and diarrhea. The study was terminated after 35 patients. It did demonstrate that FUdR did have the capacity to increase the proportion of incorporation of IUdR; however, the FUdR infusion required a decrease in the total IUdR used, and appeared to be much more toxic.

Project Description

Professional Personnel Engaged on the Project:

R. Klecker	Technician	CPB, NCI
P. Spaeth	Research Associate	CPB, NCI
J. Collins	Senior Investigator	CPB, NCI
D. Wright	Senior Investigator	SN, NINCDS

Objective: This was a phase I/II study designed to assess the toxicity and treatment results using IUdR as a radiosensitizer given as a continuous intravenous infusion for 24 hours a day combined with hyperfractionated radiation. In addition, FUdR was used as well in an effort to increase the IUdR incorporation within tumor cells by means of blocking *de novo* thymidine synthesis. The pharmacokinetics were studied in collaboration with the Clinical Pharmacology Branch.

Methods Employed

Patients with histologically confirmed glioma and other patients with locally advanced non-CNS tumors were eligible for this phase I/II study. Eligibility criteria included normal blood counts, normal renal function, and a life expectancy of at least 2 months. Patients had to be ambulatory, and had to be able to be treated as outpatients. The IUdR and FUdR were infused using a flexible silastic catheter placed in the superior vena cava. A portable automatic infusion pump maintained a continuous infusion over a 24-hour duration. Twice-a-day radiation therapy was begun within a week of the IUdR/FUdR infusion.

Major Findings

The maximum tolerable dose of IUdR had to be reduced to no more than 675 mg/m^2 per day, whenever a dose in excess of 1.2 mg/day of FUdR were given. Thrombocytopenia was the major hematologic toxicity, although glucopenia was also seen. Bone marrow recovery occurred within approximately 2 weeks after stopping the infusion. Severe mucositis was seen in almost half the cases, and diarrhea in one-third of the cases. In addition, elevated liver function tests were seen in 15-35 patients, and jaundice was seen in 3. The relationship of IUdR and FUdR is such that the amount of IUdR must be progressively reduced to accommodate increasing doses of FUdR. Thus, although there was an increased proportion of the infused IUdR that was incorporated into DNA, the total amount was not an improvement because of the reduction in the IUdR dose. This combined with the increased toxicity led us to terminate the study.

Significance to Biomedical Research and the Program of the Institute

These studies on halogenated pyrimidine analogues as clinical radiosensitizers have been useful at opening up new avenues for improving radiation responses and traditionally poorly responsive tumors.

Proposed Course

The present study has been terminated. We have now begun randomized studies of IUdR alone, given as a 24-hour infusion in conjunction with hyperfractionated irradiation therapy in the treatment of unresectable sarcomas.

Publications

1. Jackson D, Kinsella TJ, Rowland J, et al. Halogenated pyrimidines as radiosensitizers in the treatment of glioblastoma, J Clin Oncol 1987;10:437-443.

2. Kinsella TJ, Collins J, Rowland J, et al. Pharmacology and phase I/II study of continuous intravenous infusions of iododeoxyuridine on hyperfractionated radiotherapy in patients with glioblastoma multiforme, J Clin Oncol 1988;6:871-79.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06360-05 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Radionuclide Generators to Produce the Iridium-194 Beta Emitter

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Saed Mirzadeh	Expert	ROB, NCI
Others:	Otto A. Gansow	Senior Investigator	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Inorganic and Radioimmune Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The project investigates the design, manufacturing, testing, and use of a novel radionuclide generator for biomedical applications. The generator has a long shelf-half-life, many years, and produces a 20-hour radionuclide daughter which emits high-energy beta particles that have suitable characteristics for labelling proteins through bifunctional chelates.

In the 194-Os/194-Ir generator containing the parent nuclei, 194-Os, has a half-life of 6.0 years which beta-decays, EmaxB=100 KeV, to the 19.15-hour 194-Ir daughter. The 194-Ir daughter decays with the emission of 2.2 MeV beta particles to the ground state of 194-Pt (86%) and to the 328.5 KeV first excited state with emission of 1.9 MeV beta particles (9.2%). There is a 328.5 KeV first excited state with emission of 1.9 MeV beta particles (9.2%). There is a 328.5 KeV gamma-ray which follows the decay of 194-Ir with 13% absolute aboundance. The absence of high intensity gamma-rays in the decay of 194-Ir, with the exception to the 328.5 KeV, makes this beta emitter nuclei very attractive from the point of view of dosimetric considerations. On the other hand, the presence of 328.5 KeV gamma-rays makes 194-Ir a superior nucleus to 90-Y for tumor imaging.

Preliminary calculations indicate that several mCi of the parent, 194-Os, can be produced in a nuclear reactor by double neutron capture of an 192-Os (natural aboundance of 41%) target. By using enriched 192-Os, a two-fold increase in the yield results and also reduces the production of impurities. The enriched Osmium-192 with enrichment factor of greater than 99% is purchased from Oak Ridge National Laboratory.

Project Description

Objective: To develop a radionuclide generator system which will produce 194-Ir for attachment to proteins through bifunctional chelations.

Methods Employed

Osmium-194 is produced in a nuclear reactor by irradiating enriched Os-192. After irradiation, Os target is dissolved, purified and loaded into a suitable chromatographic column. The daughter 194-Ir is eluted with suitable solvents. The yield of the elution of the daughter, the breakthrough of the parent, radiation and chemical resistance of the column generator, radiochemical, and radionuclidic purity of the product are being investigated.

In addition, to test the integrity of the quartz ampule target containers in high radiation field, a series of preliminary irradiation were performed at Brookhaven National Laboratory High Flux Beam Reactor. Several blank quartz ampules were irradiated at the core of the reactor for 21 days (duration of the reactor cycle). No chemical fatigue so far has been observed. The irradiation of osmium targets are scheduled for the Fall of 1988.

Significance to Biomedical Research and the Program of the Institute

The development of the 194-Os/194-Ir generator for production of 20-hour 194-Ir would increase access of the biomedical community to a high-energy beta emitter as a radiotherapeutic agent.

Proposed Course

1) Development of 194-Os/194-Ir generator.

2) Prepare for suitable bifunctional chelates for attachment of 194-Ir for proteins.

3) Study proteins labeled with 194-Ir *in vitro* and *in vivo*.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06361-04 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)

Phototherapy of Intracavitary Spaces

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator*) (*Name, title, laboratory, and institute affiliation*)

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	J. B. Mitchell	Senior Investigator	ROB, NCI
	H. Pass	Senior Investigator	SOB, NCI
	P. Smith	Senior Investigator	BEIB
	W. Frauf	Senior Investigator	BEIB
	C. Black	Associate	ROB, NCI
	E. Bernstein	Associate	ROB, NCI

COOPERATING UNITS (*if any*)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7	7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The use of hematoporphyrin derivative and other photosensitizing agents in combination with light activation is currently being investigated as an anti-tumor modality for the treatment of intraperitoneal and intrathoracic tumors. A major advantage of this modality is the apparent selective retention of the sensitizing dye within tumors. A murine ascites ovarian carcinoma and a human ovarian tumor that grows as an ascites tumor has been used to study the characteristics of drug distribution in the peritoneal cavity. Likewise, murine models have been used to study the tolerance of the thoracic cavity structures to the phototherapy techniques being explored. The limitations of the murine model has required the extensions of the investigation to the canine model for evaluation of the toxicity of Phototherapy. Different wavelengths of light, different laser delivery systems, different sensitizers, different doses of energy, different modes of drug administration, and different monitoring devices are being studied. We have shown that Phototherapy can be used to effectively treat a murine ascites tumor. We have also shown that in both the murine and the canine model, the peritoneal serosal surface is tolerant of at least 0.5 J/cm2 and that this work can be extended to human subjects. In the murine system, we have shown that the thoracic cavity, like the peritoneal cavity, is exquisitely sensitive to treatment with red light (630 nm). The dose rate must be controlled to minimize heat build up (less than 150 mW fiber output from a forward projecting optical fiber is usually tolerated). We are exploring the use of photoimmunotherapy as an additional means of drug delivery. We are exploring the use of chemiluminescence as a means of light delivery to the cavitary spaces.

Project Description

Objective: To establish a laboratory model for treatment of intracavitary malignancies that spread by implanting on the serosal surfaces of organ such that Hematoporphyrin derivative and other photosensitizing agents can be used in combination with non-ionizing radiation. To determine the best means of delivering light and sensitizer and to establish means to better quantitate light delivered to the tumor and normal tissue (dosimetry), to originate means of improving or circumventing phototoxicity to normal tissue, and to provide means to remove viruses outside of cells or cells containing viruses either inside the cell or incorporated into the human DNA in banked blood intended for human use are secondary objectives of the study.

Methods Employed

Two different murine (thymic and athymic) systems and a canine model are being used to investigate the peritoneum for Phototherapy. For the study of the chest cavity, murine and canine models are being studied. Response, survival, histopathology are used for evaluation. In vitro cell culture techniques are being used to judge the initial effects of different sensitizers. Both pleiotropic drug resistant cell systems as well as more conventional cell models are being used. Fluorescence spectroscopy is being used to study drug administration routes as they impact on tumor localization and normal tissue distribution. Light dosimetry is being studied by photodiode placement and computer modelling and analysis. Monoclonal antibodies are being affixed to either Porphyrin C or hexaethylmethylporphyine-3-propionic acid because the sensitizers can be purified to homogeneity, have desirable absorbance characteristics, and provide different chemical means of attachment. Antibodies being studied are directed against either human lung or ovarian tumors that have been developed for growth in an athymic murine model system. General searches for sensitizers that absorb light at longer wavelengths (>600 nm) are being sought that also have the characteristics of being lipid membrane permeable and favorably partition to nucleic acid oligomers. Such sensitizers are investigated for viricidal effect.

Major Findings

For a murine model of an ovarian ascites tumor, phototherapy is effective (85% long-term survival) when using HPD and green light. Red light cannot be used in a murine model of intra- cavitary treatment because the dye is retained in liver and red light can penetrate the small size of a murine liver. Dogs tolerate one, two, and three treatments of intraperitoneal light therapy after iv and ip administration of HPD. Dose rate and total dose of light impact on tumor response. Initial work in a cell culture system that has been pretreated with HPD shows that chemiluminescence agents provide enough light to be effectively used as a light source. Plastic models of a canine thoracic cavity suggest that intralipid (fat emulsion) can be used for real-time simultaneous equal light distribution to the pleural surface when three or more fiber sources are concurrently used. Pleiotropic drug resistant CHO cells (C5) are slightly more resistant than the wild type. The resistance correlates with the decrease in cellular concentration of sensitizer. Pleiotropic drug resistant human mammary carcinoma cells are no more resistant than the corresponding wild type. HTLV-III viral particles can be rendered non-infectious by HPD and light. Histamine antagonist, both H-1 and H-2, can be used to decrease the dermal photosensitivity caused by HPD.

Significance to Biomedical Research and the Program of the Institute

The ROB is involved in clinical use of Phototherapy and this work is being applied to guide choice of tumors to treated, the dosing of light to be used, and the best means of administering sensitizer.

Proposed Course

Continue to explore the models outlined above to improve the use of Phototherapy in the clinic.

Publications

1. Manyak MJ, Harrington FJ, Glatstein E. A cystoscope holder/surgical retractor that really works, J Urology 1987;138: 105-106.

2. Manyak MJ, Matthews DM, Smith PD, Nochomovitz LE, Glastein E, Russo A. Response of normal canine ureter to photodynamic therapy using a cylindrical fiber, J Urology, 1988;139: 199-203.

3. Manyak MJ, Russo A, Glastein E. Photodynamic therapy, J Clin Oncology, 1988;6: 380-391.

4. Manyak MJ, Smith PD, Harrington FS, Steinberg SM, Glatstein E, Russo A. Protection against dihematoporphyrin ether photosensitivity. Photochemistry and Photobiology (in press).

5. Manyak MJ, Matthews DM, Smith PD, Nochomovitz LE, Russo A. Photodynamic therapy: Response of normal canine urether using a cylindrical fiber. Laser in Surgery and Medicine, (in press).

6. Russo A, Mitchell JB, Pass HI, Glatstein, E. Photodynamic therapy. In: DeVita V, Hellman S, Rosenberg S , eds. Principle and practice of oncology. Lippincott, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06363-05 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
DNA Damage by Alkylating Agents and Their Repair in Human Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. J. Fornace, Jr. Senior Investigator ROB, NCI

COOPERATING UNITS *(if any)*
Applied Genetics, Freeport, New York (Dr. D. Yarosh).

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.2	.1	.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

R. S. Day, III, D. Yarosh, and others have shown that approximately 20% of human tumor lines and viral transformed lines are hypersensitive to alkylating agents due to an apparent absence of alkylguanine alkyltransferase. This enzyme removes alkylation damage at the 0-6 position of guanine but not at other sites in DNA. Dr. D. Yarosh in collaboration with our unit has been able to partially purify the enzyme from human liver and raise polyclonal antibodies to this protein. Studies have been initiated to develop monoclonal antibodies to this protein. With high affinity antibodies, we will be able to further purify this protein. With sufficient purification, partial amino acid sequence can be obtained and used to synthesize oligonucleotide probes which will be used to screen human liver cDNA libraries. Such antibodies can also be used to screen human liver cDNA expression libraries.

Project Description

Objective: To purify the human alkylguanine alkyltransferase enzyme and ultimately isolate the gene.

Methods Employed

Standard molecular biology and protein biochemistry approaches.

Major Findings

Polyclonal antibodies to this protein have been isolated and efforts are directed at isolating high affinity polyclonal and monoclonal antibodies.

Significance to Biomedical Research and the Program of the Institute

An understanding of this defect which occurs in approximately 20% of all human tumor lines would have obvious importance in both carcinogenesis and cancer treatment.

Proposed Course

See summary.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06365-05 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
cDNA Cloning and Characterization of Genes Induced by Hyperthermia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. J. Fornace, Jr.	Senior Investigator	ROB, NCI
Others:	I. Alamo	Microbiologist	ROB, NCI
	M. C. Hollander	Microbiologist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prokaryotic and eukaryotic cells respond to environmental stress by the induction of a variety of stress-related proteins. In mammalian cells, the most well-characterized group of stress proteins are induced by hyperthermia. Transcription of heat shock proteins increases markedly after hyperthermia, and several of these genes have been cloned from human and mouse (hsp70 only) cells in other laboratories. It is likely that transcription of other genes is also induced in mammalian cells since approximately 10-20 genes are induced in prokaryotes and lower eukaryotes. We have isolated cDNA clones coding for over 20 different heat shock-induced RNA in Chinese hamster cells by differential hybridization screening of a cDNA library which we constructed from heat shock-treated cells. Based on homology to human heat shock cDNA clones isolated by L. Weber, some of our cDNA clones were found to code for hsp70, hsp27, hsp60, hsp89α, and hsp89ß. Except for hsp60, all these genes were coordinately induced by heat shock. By DNA sequencing the 2 most abundant isolates were found to code for ubiquitin and B2 RNA polymerase III transcripts. Ubiquitin plays many important roles in eukaryotic cells, including a prominent role in the removal of damaged proteins. Ubiquitin has been found to be induced by heat shock in yeast and chicken cells, but ours is the first demonstration in mammalian cells. We estimated that up to 10% of the mRNA in heat shock-treated cells were ubiquitin transcripts. We have found also that ubiquitin transcription was strongly induced in both rodent and human cells by alkylating agents which indicates a possible role for ubiquitin in the cellular response to such damage; in yeast, the RAD6 DNA repair gene is an ubiquitin-conjugating enzyme. These studies have been extended to other heat shock genes and we have found that hsp27, in particular, was induced by certain DNA-damaging agents. In the case of B2 RNA, we constructed size-selected libraries from untreated and heat shock-treated cells, and isolated 200 B2 cDNA clones. By DNA sequencing, the heat shock-inducible B2 transcripts consistently differed from those of unheated cells.

Project Description

Objective: To identify genes induced by hyperthermia in Chinese hamster cells, and to study their regulation.

Methods Employed

Standard molecular biology approaches and hybridization subtraction.

Major Findings

We have isolated cDNA clones coding for many of the transcripts induced by heat shock in Chinese hamster cells. These genes were coordinately regulated by heat shock. Maximum induction of transcription of these genes correlated with the induction of thermotolerance (cellular resistance to cytotoxicity of hyperthermia). Some were induced also by DNA-damaging agents which indicates a possible role for certain heat shock proteins in genotoxic stress. We have demonstrated for the first time in mammalian cells that ubiquitin RNA is a major heat shock-induced transcript. We have found that RNA polymerase III transcription of the B2 repetitive genetic element was induced by heat shock. By sequence analysis, the B2 genes induced by heat shock differed from those constitutively expressed.

Significance to Biomedical Research and the Program of the Institute

A more thorough understanding of the response of mammalian cells to hyperthermia would benefit both clinical hyperthermia research and also how mammalian cells respond to environmental stress. In the field of experimental hyperthermia, much of the cell biology has been with Chinese hamster cells; our cDNA clones can now be used to study these events at the molecular level.

Proposed Course

The response in mutant cells with altered sensitivity to hyperthermia will be studied. Study of the induction of these genes after different types of stress, such as oxidative stress, will be continued.

Publication

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06369-05 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radiation Characteristics of the Scanditronix MM-22 Medical Microtron

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	J. van de Geijn	Radiation Physicist	ROB, NCI
	B. Chin Arora	Clinical Physicist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.5	OTHER 1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Physics Section is continuing studies of the radiation characteristics of the Scanditronix MM-22 medical Microtron. Current research is centered around our Intraoperative Radiotherapy Program (IORT) and concerns the dosimetry of special IORT applicators. Associated with this is a study of the effects of using asymmetric electron collimation on the dose distributions of these applicators. A new color camera television system for viewing the intraoperative portal is under development. This system will employ a permanent mirror of aluminized Mylar instead of the current retractable glass mirror. This should allow remote viewing of the radiation portal during treatment.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Objectives: The optimization of the radiation and operational characteristics of the Scanditronix MM-22 Microtron.

Methods Employed

The high quality and versatile radiation measurement systems available to the Branch are used by staff personnel in cooperation with experts from the manufacturer to determine the basic performance of the various critical functions of the machine and its monitoring equipment. Several of these functions have been found less than optimal for the special purposes envisioned by the ROB and in several cases, dramatic improvements have been obtained already.

Major Findings

Several performance characteristics were unsatisfactory for our purposes: electron depth dose distribution and transverse beam profiles, monitor characteristics. Interaction between representatives of our staff and experts from the firm have resulted already in performance characteristics much better than required by the specifications.

Significance to Biomedical Research and the Program of the Institute

The Microtron is to be utilized primarily for intraoperative radiotherapy. As such, its reliability and (especially) its beam characteristics are of critical importance to this program. Also, the machine offers some technical features such as independently adjustable collimator jaws, which are meaningful only with more than minimum performance characteristics.

Proposed Course

The Microtron is undergoing a major rebuild following an accidental flood, which destroyed the electronic control systems. This rebuild also incorporates a significant electronics upgrade in which control of all beam handling and dosimetry subsystems is performed using a programmable power controller. Upon completion and acceptance testing, the value of this automated control approach will be extensively studied. Research will then continue on the technical aspects of the Microtron as a radiotherapy accelerator.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06370-04 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optimization of Treatment Planning for Brain Implants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	Huchen Xie	Computer Specialist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH Radiation Oncology Branch

SECTION Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This essentially is a continuing project, guided by the continuing experience of the medical principals and the continuing development especially in computing. The purpose of this work is to optimize physics, computer based and technical support for a brain implant protocol. In particular, it aims at optimization of the chain of procedures comprising patient data acquisition, treatment planning, including optimal delineation of the target, determination of the number of radioactive sources, their strength and position, and determination of the surgical mechanical positioning devices and the further development and adaptation of locally developed computer programs, with emphasis on versatile imaging as a basis for optimization of irregular implants.

Project Description

Personnel:

T. Delaney	Radiotherapist	ROB, NCI
D. Wright	Neuro-Surgeon	SN, NINCDS

Objective: To develop a computer-assisted system for optimization of the physical and technical aspects of radioactive seed implants in brain tumors; criteria are:

1. Accurate fitting of a critical dose rate surface around the chosen target volume, which may be a regular or irregular shape

2. A uniform dose distribution inside the target

3. A minimum number of catheters positioned in the brain in the most economical and accurate way.

Methods Employed

1. Mathematical/Physical methods are employed to develop a generalized approach to seed placement and relative seed strength. The effort now concentrates on irregularly shaped tumors.

2. Computer-based image manipulation of diagnostic CT data to determine optimal access routes. Current developmental efforts are directed toward implementation on a Mac II.

3. Development of mechanical positioning and directioning devices for use in data acquisition and in surgery.

Major Findings

Major results have been reached in the mathematical optimization, which is partly for practical clinical reasons, in part computerized, in part manual-based, so that a semi-automatic interactive computer based system will shortly be available. Considerable work needs to be done to make the system universal. An atlas is available for regular-shaped implants.

Significance to Biomedical Research and the Program of the Institute

The system is essential to technically and physically safe and radiation-economic patient treatment. The development aims at general applicability. The theoretical and computer related procedures apply to implants in general.

Proposed Course

Continuation.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06374-04 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Radiosensitizers and Radioprotectors on DNA Damage Produced by X-rays

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. J. Fornace, Jr.	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

T. Kinsella, Univ. of Wisconsin, Madison, WI

LAB/BRANCH
Radiation Oncology Branch

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Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.1	.05	.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have completed studies with the halogenated pyrimidine radiosensitizers, IUdR and BUdR. IUdR is currently in clinical trials as a radiosensitizer. This agent sensitizes cells to radiation after incorporation into cellular DNA in place of thymidine. We have previously shown that the yield of DNA strand breaks was increased in cells containing IUdR after x-irradiation. Current models of IUdR and BUdR radiolysis predict that only single strand damage should be produced in DNA. However, the lesion most important in x-ray lethality is probably DNA double strand breaks. We have found that radiolysis of IUdR in DNA of cells x-irradiated leads to mobile reactive intermediates which damage both the strand containing the IUdR and also the complementary strand which did not contain IUdR in these experiments. IUdR-induced strand breaks were almost as frequent in the unsubstituted strand as in the substituted strand. There was also a smaller increase in strand breaks in unsubstituted duplex DNA in cells containing IUdR-DNA. In cell survival experiments, cells undergoing only 1 doubling with IUdR showed almost as much radiosensitization as cells undergoing 2 doublings where both strands were substituted.

Cellular radiosensitivity is affected by many factors which may be clinically important. For example, cellular oxygen concentration and the redox potential of the cell affect cellular radiosensitivity and probably are also important clinically. Several agents, BSO or SR 2508, have been shown to decrease the effective sulfhydryl concentration in the cell and to act as hypoxic cell sensitizers. Addition of cysteamine, on the other hand, protects cells x-irradiated under oxic conditions. Previously, we have shown by alkaline and neutral elution that the above agents altered the yield of radiation-induced DNA damage.

Project Description

Objective: To study the effect of radioprotectors and radiosensitizers on particular types of DNA damage.

Methods Employed

Alkaline and neutral elution. Standard cell culture techniques.

Major Findings

Radiolysis of DNA containing halogenated pyrimidines leads to the generation of mobile reactive intermediates which can produce double strand DNA damage, in particular double strand breaks.

Significance to Biomedical Research and the Program of the Institute

There are 2 major implications for radiotherapy:

1. Significant radiosensitization can be achieved in cells undergoing only 1 round of replication in halogenated pyrimidines. Therefore, efforts should be directed toward labelling as a high a fraction of the tumor cells as possible even if many have only been labelled on 1 DNA strand.

2. DNA double strand breaks are probably responsible for much of X-ray cell lethality. We infer from our studies that the use of maximum achievable concentrations of IUdR may be preferable, since it will produce the greatest density of intermolecular damage (double strand breaks) with radiation.

Publications

None

Proposed Course

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06377-03 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (*80 characters or less Title must fit on one line between the borders.*)

Optimization of Dose Distributions from Intraoperative Applicators

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*)

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	K. Yeakel-Orr	Dosimetrist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	T. Delaney	Senior Investigator	ROB, NCI

COOPERATING UNITS (*if any*)

LAB/BRANCH

Radiation Oncology Branch

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Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project needs to be reopened for the Microtron as soon as that machine is recommissioned.

Due to the high single fraction doses employed in intraoperative radiotherapy, it is important to minimize areas of high dose. These areas arise due to the the scattering relationship between the photon collimators and the applicator system. They may be non-symmetric due to either an asymmetric applicator design, or to manufacturing tolerances of the applicator system resulting in the center of the applicator to be slightly different than the central ray of the accelerator. These dose distributions can be optimized to predetermined criteria by the use of an optimum field size for each combination of applicator and electron energy. The use of asymmetric collimators can further correct for nonuniformity in the dose distribution due to asymmetric design, applicator bevel angle, or applicator mis-alignment.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Objective: To optimize the dose distribution from applicators used to deliver intraoperative radiation therapy with high energy electron beams.

Methods Employed

1. To develop criteria for defining the optimum dose distribution.

2. To study the dose distributions from applicators as a function of collimator setting and energy in order to determine optimum field sizes for each energy.

3. To use asymmetric collimators settings to correct for non-uniformities in the radiation field due to applicator shape, non-concentric applicator position and applicator bevel angle.

Major Findings

The criteria for defining the optimum dose distribution based on the prescription isodose line have been developed. These have been used to determine optimum field sizes for the applicators currently used to deliver IORT at the NCI. The areas of high dose have been reduced by as much as 25% by this method. The use of decoupled collimators to produce asymmetric fields can restore symmetry to the dose distribution when it is affected by applicator shape or non-concentric position.

Significance to Biomedical Research and the Program of the Institute

Most institutions performing intraoperative radiotherapy use a fixed collimator setting for each applicator regardless of energy. Some places use the same field size for all applicators as well. This clearly results in areas of high dose in the treatment which can result in doses 30%-40% greater than the prescription dose. Proper choice of field size can substantially reduce these "hot spots" resulting in a more uniform dose distribution.

Proposed Course

Work on this project has been suspended pending the repair of the Scanditronix MM-22 microtron. Studies will continue as soon as repairs are complete.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06378-03 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

QA of Treatment Delivery by Means of Overlayed Digitized Simulator and Portfilms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	R. Miller	Radiation Physicist	ROB, NCI
	B. Chin Arora	Radiation Physicist	ROB, NCI
	Hunchen Xie	Computer Specialist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	Kathy Yeakel-Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

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Radiation Physics and Computer Automation Section

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1/4	1 3/4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The quality assurance of the consistency of radiation treatment delivery with the prescription is a continual concern, locally as well as nationally. The ROB already employs graticules projecting onto all simulator films and all corresponding portfilms. Now a project is being started to overlay differently processed digitized films to increase the quality of information, as well as to decrease the volume of documentation to be retained.

The system should be of great interest to inter-institutional studies as well. The project has been on hold until recently because of delays in acquisition of essential hardware. It is progressing rapidly now.

Project Description

Objective: 1) To improve the quality of documentation on the proper implementation of beam treatment set-ups.

2) To condense the amount of documentation to be kept, and to increase its objectivity and exchangeability.

Methods Employed

1. Take x-ray films at the simulator, in the planned beam positions, including graticules projected onto the films.
2. Follow similar procedure at the treatment machine, producing port films with graticules.
3. Digitize both categories of films taking care to use the same orientation, centering and magnification, with help of the graticules projected onto all films.
4. Apply appropriate computer enhancement of both simulator films and the corresponding port films.
5. Overlay technique, bring-out salient anatomical features, graticules, block delineation, etc.
6. Using computer, do measurements of significant deviations.
7. Store the results, properly labeled.

Major Findings

Exploratory experiments were very encouraging.

Significance to Biomedical Research and the Program of the Institute

1. Quality Assurance and verification will become much more efficient, self-contained and attractive to use.
2. Documentation will be much more compact and easier to use.
3. Quality Assurance of joint studies will be much easier and more objective.

Proposed Course

Pilot project to be started soon at this institution.

Publications

In preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06379-02 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phase I Study of Photodynamic Therapy for Surface Malignancies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS (if any)

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Radiation Oncology Branch

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Radiation Therapy Section

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Photodynamic therapy involves the use of a light activated compound which localizes in tumor, followed by the activation of this compound by light for cytotoxic effects for the treatment of cancer. The current protocol uses the intravenous administration of the Photofrin II preparation of the hematoporphyrin derivative, the only currently approved photosensitizer for use in humans. This is followed by the delivery of light to the affected area. Light is directed at the tumor using optical fibers coupled to a argon/pumped dye laser. Hematoporphyrin derivative selectively localizes in tumor compared to certain normal tissues. The mechanism of this is not well understood at this time, but has been documented in both animals and in patients. Selective retention of the photosensitizer in combination with focal light delivery to the involved area permits selective destruction of tumor with minimal effect on uninvolved normal tissue. Hematoporphyrin-derivative photodynamic therapy may be clinically useful in a number of anatomic sites involved by tumor.

Project Description

Professional Personnel Engaged on the Project:

H. Pass	Senior Investigator	SB, NCI
W. Sindelar	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIB, DES
R. Bonner	Biophysicist	BEIB, DES
P. Smith	Laser Physicist	BEIB, DES
W. Travis	Senior Investigator	LP, NCI
A. Dwyer	Senior Investigator	RB, NCI

Objective

This a Phase I study designed to assess the toxicity and effectiveness of photodynamic therapy with Photofrin II and laser light in treatment of surface malignancies in patients with neoplasms that have failed to respond to previous treatment or for whom no Phase II or III agents or studies are available. The responsiveness of different malignant tumors at various anatomic sites to treatment with photodynamic therapy is also being studied. Physical parameters of light distribution in tissue are being measured, as well as pharmacology to assess the localization of photosensitizer in tumor, in order to correlate these measurements with treatment effects from photodynamic therapy. Changes in blood flow induced by photodynamic therapy are being studied in patients and laboratory animals using laser doppler blood flow velocimetry. Efforts are under way to develop technical expertise to measure the production of singlet oxygen, which is thought to be the active cytotoxic moiety produced by the interaction of light-activated photosensitizer and oxygen, in hopes of correlating this with the responsiveness of tumors to photodynamic therapy.

Methods Employed

Patients with surface malignancies that have or have not had previous treatment with other agents are eligible for this protocol. Included are patients with any malignancy on or just underneath the surface of the skin (primary or metastatic), surface tumors of the gastrointestinal tract, surface tumors within the tracheal and bronchial tree, surface genitourinary tumors of females, and malignant pleural or ascitic tumors stemming from the surface of the chest wall or peritoneum. Patients with porphyria of any type, severe liver disease, or a curative option for the stage of disease are not eligible for treatment. Patients receive their Photofrin II photosensitizer by intravenous administration 1.5 - 2.0 mg/kg. Light is then delivered in single or multiple fractions to the involved tumor area, using optical fibers coupled to an argon dye laser. Optical fibers are either used for surface illumination, endoscopic treatment, or intraoperative treatment, depending on the patient's clinical problem.

Major Findings

Thirty-seven patients have been entered on the protocol. Twenty-three patients have had recurrent carcinoma involving the skin, 18 with recurrent breast cancer on the chest wall. Of the patients with recurrent cancer on the chest wall, the first 4 patients were treated to symptomatic areas only. The next 4 patients were treated to all visible disease with a single fraction of light 72 hours after hematoporphyrin injection. One of these patients had a complete clinical and pathologic response, but had necrosis of skin heavily involved by tumor requiring resection and grafting. Because of concern about tumor necrosis from the single fraction light, the subsequent 10 patients with recurrent breast cancer have been treated with multiple fractions of light (2 to 3 fractions of light 48-96 hours after hematoporphyrin administration, with escalating doses of light in an attempt to determine the optimum dose and treatment schedule. Response on these patients is pending. Other patients treated for skin malignancies include 2 patients with recurrent skin cancer of the head and neck, one of whom had complete responses in seventeen treated lesions and a second patient who was lost to follow-up because of progressive disease; and one patient with cutaneous lymphoma in whom 9 lesions had a complete response and 3 lesions had partial responses. Pigmented melanoma treated in one patient did not respond because of heavy pigmentation which attenuated light. One patient with epidemic Kaposi's sarcoma has been treated with 2 courses of hematoporphyrin derivative and light. The reponse to treatment is pending. Nine patients have been treated for endobronchial obstruction. Treatment was considered effective in 8 of 9 patients, with either relief of bronchial obstruction or maintenance of narrowed but patent airway. One patient with recurrent esophageal cancer at the site of his pharyngeal anastomosis, showed a partial response to treatment after 2 courses of treatment. Four patients with recurrent ovarian cancer have received hematoporphyrin derivative. Three of four patients were able to undergo resection of disease and light delivery to the entire peritoneal surface at the time of laparotomy. Light doses have been sequentially escalated from 0.2 to 0.3 to 0.4 J/cm^2. Follow-up times are 0, 1, and 6 months in the 3 adequately treated patients. In the first patients treated, previously positive peritoneal washings cleared or remained so for 5 months, when the patient developed progressive nodal disease in the pre-sacral area. Peritoneal washings on the second patient have become negative 1 month after therapy. Morbidity to date includes grade 1 elevation of serum liver enzymes after hematoporphyrin derivative administration in 1 patient with Aids/Kaposi's sarcoma and a history of hepatitis who is on AZT. Liver function tests returned to baseline six weeks after photosensitizer injection. Four patients have developed cutaneous photosensitivity (first degree sunburn on the arms or the hands) upon exposure to light within 4 to 6 weeks after drug administration. Among patients treated for skin problems, 1 patient developed full thickness skin necrosis in the treatment field requiring surgical repair. Patients treated to extensive chest wall fields have experienced moderate discomfort in the treatment field, for 48-72 hours after light delivery, which has been relieved with P.O. narcotics.

One patient with extensive ulcerated recurrent breast cancer did require intravenous narcotics for 24 hours for pain control after treatment. Superficial skin breakdown has been seen in 2 patients with recurrent inflammatory carcinoma involving the entire chest wall after photodynamic therapy. Areas of extensively involved skin superficially ulcerated and subsequently formed eschars which when on to heal after appropriate skin care. One patient with recurrent nodules of carcinoma involving the skin of the face and neck developed mild facial edema after treatment. In the patients with bronchial lesions, 1 patient treated for obstructing recurrent cancer of the right mainstem bronchus died of massive hemoptysis from recurrent tumor into the right maintem bronchus 3 months after treatment. The contribution of photodynamic therapy to this complication is undetermined. One patient treated for segmental bronchial obstruction developed a radiographic infiltrate after treatment. This resolved on antibiotics. One patient who underwent photodynamic therapy for endobronchial metastases developed a transient elevation of non-cardiac creatine phosphokinase after treatment, significance unclear. In patients treated to the peritoneal cavity, 1 patient developed cutaneous erythema and subsequently mild hyperpigmentation of the skin of the abdominal wall which was exposed for a long period of time in the operating room lights. We have modified the operating room lights with filters to reduce the risk of this problem.

To date then, we have determined that photodynamic therapy has activity against a variety of malignant tumors, even after extensive previous treatment. We have developed a method for delivery of photodynamic therapy to the skin, bronchus, peritoneal cavity (abdomen), and esophagus. Information on toxicity and response to treatment are encouraging to date.

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially curative therapy for selective groups of patients with malignant disease. In particular, patients with a tumor that is accessible to light either by superficial, endoscopic or interstitial illumination may benefit from treatment. Intraoperative treatment is both practical and potentially efficacious. We envision a possible use for this therapy in patients with carcinoma in situ of the urinary bladder, tumors involving the peritoneal and pleural surfaces, and in selected skin cancers.

Proposed Course

This protocol is approved for treatment of 50 patients. Depending on the results of the patients treated, we hope to move on to Phase II studies in the following sites: abdomen (peritoneal cavity), bronchus, and esophagus. We would be interested in testing the efficacy of photodynamic therapy in patients with refractory pleural effusions and patients with pleural malignancies. Animals studies are currently in progress as a prelude to such clinical work. Long-range plans also include examination of other photosensitizers which may be activated by light with deeper tissue penetration, and which may have less cutaneous photosensitivity.

Publications

1. DeLaney TF, Glastein E. Photodynamic therapy of cancer, Comprehensive Therapy 1988;14:43-55.

2. DeLaney TF. Treatment of cancer with light activated compounds (editorial), J Clin Onc 1987;5:1714-5.

3. DeLaney TF. Photodynamic therapy. In: McGrath I, ed. New directions in cancer treatment, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06380-02 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)
Molecular Biology of Cellular Injury

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. J. Fornace, Jr.	Senior Investigator	ROB, NCI
Others:	M. Papathanasiou	Visiting Fellow	ROB, NCI
	I. Alamo	Microbiologist	ROB, NCI
	M. C. Hollander	Microbiologist	ROB, NCI

COOPERATING UNITS (if any)
Dr. Nikki Holbrook, NIA, Baltimore, MD; Dr. Daniel Nebert, NICHHD
Dr. Ian Hickson, University of Newcastle on the Tyne, U.K.
Dr. Louis Barrows, University of Utah, Salt Lake City, UT

LAB/BRANCH
Radiation Oncology Branch

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.4	0.4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

In bacteria and yeast, many of the genes important in the cellular response to DNA damage are induced by such damage. Based on observations in bacteria and yeast, we predicted that, if similar responses occur in mammalian cells, such genes will have these characteristics: 1) low abundance transcripts; 2) rapid induction; 3) induction of only 2-10 fold; 4) induction specific for DNA damaging agents. In order to isolate cDNA clones for DNA-damage-inducible (DDI) transcripts which may be induced only several fold, we used hybridization subtraction at low ratios of RNA:cDNA. With this approach, 49 different cDNA clones were isolated which coded for low abundance transcripts that were rapidly induced by the DNA-damaging agent, UV radiation, in Chinese hamster cells. Most were only induced by DNA damage and not other types of stress such as heat shock. The cDNA clones were divided into 2 general classes; in the first, induction occurred only after treatment of cells with UV or UV-mimetic agents, while in the second class, induction was seen with many agents which produce high levels of DNA base damage such as alkylating agents. By DNA sequence analysis most of our cDNA clones did not correspond to any known genes. One cDNA clone, *DDIA18* had 2 localized areas of homology with a known DNA single strand binding protein. Another of our cDNA clones, *DDIA153*, was found to code for RNA which was increased either by DNA damage or inhibition of cell growth. *DDIA153* may code for a growth cessation gene which could have a protective effect on cells exposed to DNA-damaging agents. In collaboration with I. Hickson and L. Barrows, expression of our DDI transcripts in DNA repair mutants has been investigated. Several examples of both increased and decreased expression transcription in the mutant cells has been observed. Increased expression of certain DDI genes have been found in C-14Cos mouse liver in collaboration with D. Nebert; this mutant contains a deletion in chromosome 7 which may regulate one or more stress responses. The functions of most of our DDI sequences are unknown, but it is probable that the protein products of at least some of these transcripts play a role in the cellular response to DNA damage.

Project Description

Objective: To isolate cDNA clones which code for transcripts induced by DNA-damaging agents in mammalian cells.

Methods Employed

Standard molecular biology techniques and specialized hybridization subtraction cDNA cloning approach which was developed in this laboratory.

Major Findings

Forty-nine different cDNA clones were isolated which code for different mRNA induced by UV radiation. Most of these cDNA clones coded for transcripts which were specifically induced by DNA damaging agents. These genes have been well conserved (which suggests important functions) since they were expressed and induced by UV in human cells. Based on DNA sequence analysis and other studies, *DDIA18* was found to code for a nucleic acid single strand binding protein which is DNA-damage inducible. The human equivalent of DDIA18 has been isolated and sequenced; this protein is very highly conserved in humans and rodents. Several examples of abnormal expression were found in DNA repair mutants.

Significance to Biomedical Research and the Program of the Institute

DNA damage and its repair play a central role in carcinogenesis and also in the cellular response to many antineoplastic agents. Since our clones code for genes induced by DNA damage, it is likely that their protein products play a role(s) in the response of cells to this type of injury.

Proposed Course

A high priority is to determine the biologic importance of the sequences isolated. One approach is to study their expression in mutant cells as already described. The possibility exists that our clones may code for the mutated gene in these different cell lines. For fine mapping studies, full length cDNA clones need to be isolated. We plan to isolate full length clones from both a Chinese hamster and a human library. With the human clones, we can effectively study their expression in spontaneous mutants such as xeroderma pigmentosum. With full length clones, expression vectors can be constructed to determine if a particular sequence reverses the mutation in the mutant cell lines described above. In the case of *DDIA153*, we have already isolated and sequenced a full length clone in collaboration with N. Holbrook; studies have been initiated with recombinant expression vectors.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06381-02 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Modeling of Time-Dose Response of Human Tumors and Normal Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	J. Mitchell	Radiobiologist	ROB, NCI
	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH Radiation Oncology Branch

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TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In clinical radiotherapy, the choice of the fractionation parameters: total time, number of fractions, dose per fraction, time-spacing of the fractions, is still largely based on experience. The present project develops a formalism to describe the normal tissue reactions to irradiation regimens over time in terms of the (indirect) reactions of the functioning of normal tissue in relation to the reaction to radiation damage to the stem cells or transition cells. Normally there is a steady state of regular replacement of the normal attrition of functioning cells. This equilibrium condition is upset by radiation lethal damage to sensitive stem cells and transition cells. Full or tolerable recovery of functionality depends on the level of damage to the stem cell population, the distribution of damage over time, and on the tissue kinetic characteristics. Similar considerations apply to the tumor. The present model uses either the Linear-Quadratic or the Two-Component model for the immediate survival of vulnerable normal tissue or clonogenic tumor cells. It requires selection of tissue characteristics such as overall normal tissue turn-over time, acute stem cell doubling time, sublethal damage recovery time, and tolerance level functionality, in order to computer-generate time-dose response curves consistent with actual clinical response patterns, observed with conventional regimens. For tumors, similar selections are needed, as well as a criterion for cure rate. Successful selections are then tested with known alternative schemes. The ultimate aim is to develop reliable predictive rules for clinical radiation therapy.

Project Description

Objectives: To develop a mathematical formalism describing:

1. The attrition of functioning normal tissue cells.
2. The survival rate, per single dose, of viable stem cells.
3. The inter-fraction and post-treatment course repopulation including an account of the sub-lethal damage repair, of viable stem cells.
4. The survival rate of clonogenic tumor cells per single-dose.
5. The effective dose for early as well as late reacting normal tissues.
6. The inter-fraction and post-treatment growth pattern of the clonogenic cells, as well as the gross tumor.

Methods Employed

1. The alpha/beta (2-parameter) model as the single-hit/ single-target, single-hit /multi-target (3-parameter) model are explored for the single-dose response of stem cells and clonogenic tumor cells.
2. Linear attrition over time is assumed for functioning normal tissue cells as well as non-clonogenic tumor cells.
3. Normal tissue cell loss and replacement is under homeostatic control.
4. Clonogenic tumor cells proliferate exponentially over time.
5. Stem-cell proliferation is triggered only after some distress signal related to functionality cell levels drop below a certain threshold.
6. Normal tissue tolerance is interpreted as the lower limit of normal tissue functionality: the normal tissue functioning cells dropping below some fraction of their normal count.

Major Findings

1. A provisional mathematical model has been developed.
2. An interactive computer program has been developed which enables automatic search for acceptable parameters, based on estimates of reasonable ranges of certain key parameters, such as D0, cell doubling times, etc.
3. It is possible to simulate time-dose response patterns for certain conventional and unconventional fractionation schemes, which are reasonably consistent with published findings in some clinical trials.
4. It is possible to calculate isoeffective doses for late reacting normal tissues, as well as tumors.

Significance to Biomedical Research and the Program of the Institute

1. The present model shows promise as a tool toward understanding of time-dose response to conventional or “standard” treatment schedules, as well as some hyper-fractionation schemes and other non-standard schemes.

2. The model may be used to explore, by simulation, other unconventional schemes, and provide reasoned guidance to at least avoid work results, especially as regards to late reactions and tumor.

Proposed Course

1. Continuation of study of literature data
2. Design of relevant experiments
3. Expansion to low dose rate treatment, both single session and fractionated

Publications

van de Geijn J. Time-dose response of human tumors and normal tissues during and after fractionated radiation treatment: A new model. Radiat Therapy and Oncol 1988;**12**: 57-88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06382-02 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radiation Therapy with Radiolabelled Antibodies: Technical Aspects and Dosimetry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	E. Lamoreaux	Computer Specialist	ROB, NCI
	H. Xie	Computer Specialist	ROB, NCI
	J. Carrasquillo	Nuclear Medicine Physician	NM, CC

COOPERATING UNITS *(if any)*

Nuclear Medicine Department, CC; Diagnostic Radiology Department, CC.

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 10.0	PROFESSIONAL: 8.5	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Administration of radiolabelled antibodies is a relatively new treatment modality for certain forms of cancer. Much of this field is developmental in nature. In particular, the dosimetry of tumor masses, especially at the microscopic level, is as yet unknown. The Radiation Physics and Computer Automation Section is actively assisting in the implementation of clinical protocols. Current research is in two major areas.

Imaging of the organ-specific distribution patterns on a temporal basis is fundamental to the understanding of antibody kinetics and for large volume radiation dosimetry (at the total organ level). The ability to accurately localize biodistribution patterns using nuclear medicine imaging techniques and to accurately register these images with respect to other imaging modalities (CT or MRI) is essential for obtaining quantitative results.

Dosimetry of microscopic tumor masses is approached through the use of computer modeling. The results, where possible, will be validated using quantitative autoradiography.

Project Description

Objectives: To localize the sites of retention of radiolabelled antibodies and to determine the deposition-retention kinetics as well as the clearance pathways. To determine normal organ radiation doses and tumor dose, it possible on a microscopic level for alpha, beta and gamma emitting radionuclides. To determine the optimum combination of imaging modalities for localization and to determine the lower limits of detection of tumor masses with external imaging devices.

Methods Employed

This project will use small animal models to determine the metabolic pathways of various antibodies and their deposition-retention-excretion kinetics. Phantom studies will be conducted to determine the optimum imaging modalities and their lower limits of detection. These will be confirmed using large animal models. Computer models for determining dose distributions on a microscopic level and for alpha emitting radionuclides will be developed and tested with animal models. Patients under treatment will be imaged, as appropriate, and will be bioassayed using external counting techniques. Biopsies will be taken and used to validate metabolic and dosimetric models for each radiolabelled antibody.

Major Findings

Studies at other institutions indicate that therapy with radiolabelled antibodies offers little advantage over conventional forms of radiation therapy in the treatment of large tumor masses, due to the inhomogeneous distribution pattern of organ uptake. This results in large dose gradients within the treated site. Antibody therapy shows real promise, however, in the treatment of small tumor masses, especially microscopic disease. The problem with this approach is that the size of these masses makes them difficult to localize using traditional nuclear medicine imaging techniques. It may be possible to image these masses by employing other imaging modalities, either singly or in combination. Also, the dose calculational formalism for distributed radionuclide sources (MIRD), may no longer be valid under these conditions, since the range of the particulate radiations may be greater than the dimensions of the tumor mass and the distribution of radioactivity may be inhomogeneous. A new formalism will need to be developed for alpha emitting radionuclides, as their energy deposition pattern differs significantly from beta-gamma emitters.

Significance to Biomedical Research and the Program of the Institute

Radiolabelled antibodies are a new, exciting potential treatment modality. They offer the promise of selectively irradiating tumor masses, while delivering minimal radiation doses to normal tissues. This represents the ideal form of radiation therapy. It is possible that, for some forms of cancer, radiolabelled antibody therapy will supplant chemotherapy as the treatment of choice for microscopic disease.

Proposed Course

To be continued. The GE GEMINI SPECT-capable gamma camera originally dedicated to this project is to be replaced by a different unit, due to protracted installation and interfacing problems. A laser system to facilitate patient alignment will be installed in the room in which the new camera will be located. Phantom studies will shortly commence, first employing simple geometries and progressing to humanoid organ phantoms. Image processing techniques will be developed to correlate images from different scanning modalities to aid in diagnosis and treatment planning. Two alternative methods for quantitating whole-body clearance of gamma-emitting radioisotopes will be instituted and compared. The first uses a dedicated microcomputer with both multichannel analysis and multichannel scaling capabilities, while the latter is a much simpler, less expensive system utilizing a portable, data-logging radiation detector.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06383-02 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of an Improved Treatment Chair for Radiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Miller	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	F. Harrington	Biomed. Engineering Tech.	ROB, NCI
	J. van de Geijn	Radiation Physicist	ROB, NCI
	J. Ovadia	Visiting Scientist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is intended to design a treatment chair which overcomes the inherent design limitations of commercially available chairs. This chair will function independently of the treatment couch so as to permit opposed field treatment in any orientation in an extended isocentric fashion (the center of rotation will be at a distance greater than the standard isocenter of the accelerator). It will be capable of accurate, reproducible rotation and translation in the lateral, longitudinal and vertical planes. If possible, the chair will function with a standard radiotherapy simulator to permit proper localization, immobilization and treatment planning.

The chair is being designed on the "tool platform" principle. That is, the chair will function as a platform, allowing the attachment of various additional devices which can be placed in such a manner that they permit proper immobilization of the patient without unduly restricting treatment delivery.

Project Description

Objectives: To develop an independent treatment chair to permit multiple-field radiation therapy at either standard or extended SSD.

Methods Employed

The Radiation Therapy machine shop fabricates any chair components and accessories that are needed. Selected patients are placed in the chair for simulation and for their course of therapy. Any problems associated with immobilization and repositioning are analyzed on a daily basis and the necessary modifications are made.

Major Findings

The initial version of the treatment chair permitted opposed-field treatments and could be used with the simulator as well as with any treatment unit. Treatment of some forms of cancer with the patient seated is advantageous. The original design of the chair was excessively limited by the stipulation that it operate with the simulator. The hydraulic vertical motion was imprecise and the positioning of the patient by hand was operationally difficult. The center of rotation of the chair was also at an undesirable location, which precluded its use in an isocentric manner.

Significance to Biomedical Research and the Program of the Institute

Treatment of the mediastinum with the patient seated can minimize the amount of lung in the irradiated field, minimizing complications. A combined Waldeyer's/ mantle field treatment is possible in this position. Also, low dose rate mantle fields can be used by placing the chair at an extended SSD.

Proposed Course

Currently, the chair has undergone a major modification which incorporates a base-mounted turntable to provide isocentric positioning of the patient. This greatly simplifies the initial set-up and treatment. The vertical stability has also been greatly improved. These modifications still permit the chair to be used with our current simulator. A new elevating mechanism will be developed to give precise control over patient positioning in the vertical direction. Also, the possibility of providing motor driven controls will be explored and additional attachments for positioning and immobilizing the patient will be developed.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06384-01 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation and cDNA Cloning of DNA Polymerase ß in Chinese Hamster Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. J. Fornace, Jr.	Senior Investigator	ROB, NCI
Others:	I. Alamo	Microbiologist	ROB, NCI
	M. C. Hollander	Microbiologist	ROB, NCI

COOPERATING UNITS *(if any)*

S. Wilson, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

ß-polymerase is one of the few mammalian DNA repair genes which have been isolated. This enzyme is responsible for the repair polymerase step ("gap filling" step) of DNA repair. In the case of short patch repair after damage by base damaging agents such as alkylating agents, ß-polymerase is the only polymerase utilized. The genes of repair polymerases in E. coli and yeast have been found to be DNA-damage inducible. In collaboration with S. Wilson who has cloned both human and rat ß-polymerase cDNA, we have found that ß-polymerase RNA is rapidly induced in Chinese hamster ovary (CHO) cells after exposure of the cells to alkylating agents or hydrogen peroxide. Induction did not occur after UV radiation, heat shock, perturbation of cell cycle, or exposure to other DNA damaging agents which did not induce high levels of adducts to single bases in DNA. This is the first demonstration of the induction of a DNA repair gene in higher eukaryotic cells specifically by DNA damage. In order to further elucidate the regulation of ß-polymerase in CHO cells, we have isolated the ß-polymerase cDNA clone from a CHO cDNA library. Sequence analysis demonstrated that ß-polymerase has been highly conserved in the Chinese hamster, rat, and human species. With the Chinese hamster cDNA, we plan to isolate and characterize the Chinese hamster ß-polymerase gene with particular emphasis on the regulation of the DNA-damage inducible response.

Project Description

Objective: To study the regulation of ß-polymerase gene in Chinese hamster cells.

Methods Employed

Standard molecular biology approaches.

Major Findings

The Chinese hamster ß-polymerase gene is the first mammalian DNA repair which has been found to be specifically induced by DNA-damaging agents. We have cloned and sequenced a Chinese hamster cDNA clone for ß-polymerase.

Significance to Biomedical Research and the Program of the Institute

ß-polymerase plays an important role in the repair of damage by DNA damaging agents such as alkylating agents. Characterization of this gene and its regulation will probably provide insight into the response of mammalian cells to agents with relevance in both cancer therapy and carcinogenesis.

Proposed Course

See summary of work.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06385-01 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Increased Expression of Stress-induced Genes in Chemoresistant Tumor Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. J. Fornace, Jr.	Senior Investigator	ROB, NCI
Others:	M. C. Hollander	Microbiologist	ROB, NCI
	M. Papathanasiou	Visiting Fellow	ROB, NCI

COOPERATING UNITS *(if any)*

Dr. Thomas Hamilton, NCI; Dr. Robert Ozols, NCI
Dr. K. B. Tan, Smith, Kline, & French Laboratories, Philadelphia, PA
Dr. Regina Goth-Goldstein, University of Berkeley, CA

LAB/BRANCH

Radiation Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

A major problem in cancer chemotherapy is the emergence of drug-resistant cells. Chemotherapy-resistance is probably due to multiple mechanisms including altered uptake/excretion of drug, increased inactivation of drug, and altered host response such as increased DNA repair. As outlined in project number Z01 CM 06380-02 RO, we have isolated a variety of mammalian cDNA clones which code for transcripts specifically induced by DNA damage. We have initiated studies with a variety of human tumor cell lines which have been selected for resistance to cis-Pt diamminedichloride (DDP), nitrogen mustard (HN2), or other alkylating agents. Our initial studies have involved determining the level of different stress-induced transcripts in these chemoresistant tumor cell lines compared to their parent cell lines with normal sensitivity. When this was done with P-388 cells of the mdr phenotype, a marked increased in mdr RNA compared to the parent line was observed; probes used included the rodent cDNA isolated by Ling and the human cDNA isolated by Gottesman. None of our *DDI* transcripts were elevated in this P-388 line. In contrast, there were multiple examples of over-expression of certain of our *DDI* transcripts in DDP and alkylating agent resistant cells. For example, in the case of *DDIA33* RNA, this transcript was constitutively elevated in both DDP, melphalan, and HN2 resistant human tumor cell lines and also in a MNNG resistant Chinese hamster cell line. It should be noted that these cell lines were not of mdr phenotype and mdr RNA was not increased. The levels of different hsp (heat shock protein) transcripts were determined also and no increased expression was found. These studies raise the important possibility that over-expression of certain DNA-damage-inducible genes may play a role in chemotherapy resistance.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Objective: To determine expression of DNA-damage-inducible transcripts in chemotherapy resistant tumor cells, and ultimately their role in chemotherapy resistance.

Methods Employed

Standard molecular biology techniques.

Major Findings

The major positive findings are summarized below:

Increased Expression of *DDI* Transcripts and Other RNA in Chemoresistant Tumor Cells

		cDNA clones												
cell line	resistance	A18	A7	A15	A33	A45	A77	A88	A94	A99	A121	A153	mdr	A143
Raji	HN2	-		+	+++	-		-	++	++		++	-	-
P-388	mdr	-	-	-	-	-	-	-	-	-		-	+++	-
ovarian:														
2780^{CP}	DDP	+			+	++	++		++			-		-
413CP28	DDP	++	++	+	++			-	++	+	+	++	-	+
4ECP32	DDP	+	++	+	++			-	++	+	+	-	-	+
C30	DDP	++	-	-	-			-	+	+	+		-	-
2780^{ME}	melphalan	++	+	+	++			++	++	-	+	++	-	+
C13 (CHO)	MNNG	-	-	-	+++	-	-	-	-	-	-	-	-	-

+++: >8 fold increase relative to parent line; ++: 4-8 fold; +: 2-4 fold; -: no increase.

Significance to Biomedical Research and the Program of the Institute

Identification of genes and regulatory pathways involved in certain forms of chemotherapy resistance may lead to better understanding of this serious clinical problem, and may ultimately provide insights into overcoming this problem.

Proposed Course

The short-term approach will be to determine DDI transcript levels in a variety of chemotherapy resistant cells and attempt to correlate this with cellular resistance. The long-term course involves a thorough characterization of these genes and their regulation.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06386-01 RO

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radioimmunotherapy of Peritoneal Cancer with I-131 Labeled B 72.3

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Raubitschek	Senior Investigator	ROB, NCI
Others:	J. Carrasquillo	Head, Antibodies Project	NM, CC
	S. Larson	Senior Investigator	NM, CC
	R. Neumann	Chief	NM, CC
	J. Reynolds	Senior Investigator	NM, CC
	J. Schlom	Chief	LTIB, NCI
	D. Colcher	Senior Investigator	LTIB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

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NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5		

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In cooperation with the Department of Nuclear Medicine, and the Laboratory of Tumor Immunology and Biology, we have initiated clinical trials for the treatment of peritoneal carcinomatosis. A classical phase one study has begun using escalating doses of I-131 labeled antibody administered intraperitoneally.

We have treated a total of eight patients with therapeutic doses of antibody, the first two at 50 millicuries, the next three at 100 millicuries, and the last three at 125 millicuries.

The first two patients were heavily pre-treated with chemotherapy. They showed no response to therapy and progressed. Two of the patients receiving the 100 millicurie dose also progressed. The third patient will soon be evaluated with repeat CT, and if negative, will undergo exploration of the abdomen to document disease status.

The three patients treated with 125 millicuries are not yet evaluable.

There has been minimal toxicity of the radiolabeled antibodies with two patients at the highest dose having abdominal cramps following the instillation of antibody, and one patient had suppression of her bone marrow causing Grade I toxicity in her PMN count, and Grade II toxicity of her platelet count.

During the next year, we plan to treat additional patients at higher doses, and evaluate the use of different radioisotopes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM 06387-01 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Superoxide Dismutase Mimics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Russo	Senior Investigator	ROB, NCI
Others:	A. Samuni	Visiting Scientist	ROB, NCI
	C. Black	Associate	ROB, NCI
	C. Krishna	Associate	ROB, NCI
	E. Bernstein	Associate	
	J. B. Mitchell	Senior Investigator	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Experimental Phototherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There has been recent interest in the administration of superoxide dismutase (SOD) for ablation of oxygen mediated tissue damage. It is known that oxygen radicals such as superoxide and hydroxyl radical are important radiolysis products in an oxygenated aqueous environment. To date, there is no means of easily studying the effects of augmenting SOD cells. We have found that certain nitroxides, particularly oxazolidine containing nitroxides, may act as low molecular weight, cell permeable, superoxide dismutase mimics. We have demonstrated pH dependence of the oxazolidine nitroxide (OXANO) SOD mimic in both a Xanthine oxidase and cesium source superoxide generating systems. Similarly, the rates of SOD like activity of the oxan system has been determined. The scope of the chemistry is being investigated by synthesizing analogues. Likewise, the biochemistry of the reaction is under investigation. Hopefully, the physiology at the murine level will be studied if the in vitro cellular work indicates that the compounds are not toxic.

Project Description

Objectives: To create simple, low-molecular weight, metal-independent chemical systems which mimic the chemical behavior of superoxide dismutase. To study the role of such mimics as they might relate to radiation and chemotherapeutic drug modulating and modifying agents. To investigate reactions of the spin-trapping agent DMPO once it has reacted with superoxide anion radical and show that interpretation of such chemistry is germane to the interpretation of results relating to radiation and drug anticancer treatment.

Methods employed

Electron spin resonance spectroscopy allows the study of free radical chemistry and biology. The study of short lived oxy-radicals (spin trapping) or the rate of interaction of superoxide with oxazolidine nitroxides (stable spin labels) is well suited to the use of electron spin resonance. Organic synthesis of different oxazolidine nitroxides will follow straight forward procedures. UV, NMR, IR, and Mass spectroscopy will be used to characterize the chemical nature of the compounds. Cell culture techniques will be used to evaluate drug and radiation modulation. Immediate use of polymorphonuclear white blood cells to investigate the oxygen burst phenomenon and the interpretation of DMPO reactions, as well the use of superoxide dismutase mimics in changing the effects of the oxygen burst. Murine systems will be used to investigate the pharmacology, biodistribution, and metalism of the different nitroxides.

Major findings

DMPO-OH is degraded rapidly to a non-esr signal compound by DMPO. Such findings indicate that caution must be used in the interpretation of data relating to hydroxyl radical production when using the commonly employed spin trapping agents. 2,3,3-trimethyl-2-ethyl ox(3)azo(1)lidine nitroxide is the first example of a low-molecular weight, non- metal superoxide dismutase mimic. The reaction is dependent on both the superoxide reduction of the nitroxide and subsequent superoxide oxidation of the reduction product, hydroxyl amine. The reaction is pH dependent (optimal conditions are from 7 to 7.5) and the rate constants are in the order of 105 (lower than superoxide dismutase, but much higher concentrations are attainable; therefore, effective catalysis is higher for the superoxide dismutase mimic).

Significance to Biomedical Research and the Program of the Institute

A better and more complete understanding of the limitations of the common tools used to study ionizing radiation and chemotherapeutic drug effects on biological systems. The superoxide dismutase mimic may have applications in the area of coronary reperfusion, arthritis treatment, inflammation resolution, and decreasing harmful effects of the anthracyclines and bleomycin antineoplastic agents (respective cardiac and lung toxicities), as as well as changing the dose response to radiation induced damage.

Proposed course

To explore the breath of the chemical and biochemical reactions of the first oxazolidine nitroxide. To synthesize analogues which have different characteristics to explore the celluar effects of having superoxide dismutase activity in different sites (membrane, cytoplasm, nucleus, mitochondria) and to investigate the dose modifying effects of such superoxide dismutase mimic on ionizing radiation and chemotherapy drugs.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06388-01 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Treatment of Superficial Carcinoma of the Bladder with Photoradiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. F. DeLaney	Senior Investigator	ROB, NCI
Others:	E. Glatstein	Branch Chief	ROB, NCI
	A. Russo	Senior Investigator	ROB, NCI
	L. Dachowski	Nursing Clinician	ROB, NCI
	G. Thomas	Microbiologist	ROB, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bladder cancer is subdivided into four groups: 1) Superficial high-grade disease (TIS); 2) superficial disease (T1); 3) superficially invasive into the muscle (T2); and 4) tumor extending into deep muscle and/or perivesical fat (T3A/T3B). Standard therapy for superficial disease confined to the mucosa or submucosa consists of transurethral resection and intravesical chemotherapy (thiotepa, mitomycin C, BCG). Recurrence rates may range from 30-85% depending upon the grade of tumor and multiplicity of lesions. The concept of a full field defect in patients with carcinoma in-situ in association with a solitary papillary tumor is supported by the high incidence of invasive disease developing within two years following resection alone. Five-year survival rates for patients developing invasive disease (T2/T3A) range from 31-52%. Early control of superficial disease offers a potential advantage towards reduction of the overall death rate in bladder malignancy. Carcinoma in-situ refractory to intravesical chemotherapy is a particularly troublesome clinical entity, as patients are at high risk for the development of invasive disease and may require removal of the urinary bladder (cystectomy). Recent work with hematoporphyrin derivative (HpD) sensitized photodynamic therapy of the bladder mucosa suggests high cytotoxic effect, but low systemic toxicity. This modality may permit treatment of superficial carcinoma of the bladder as well as carcinoma in situ which may permit bladder preservation with cure of tumor.

Project Description

Professional Personnel Engaged on the Project:

W. M. Linehan	Senior Investigator	SB, NCI
C. Robertson	Senior Investigator	SB, NCI
W. Friauf	Engineer	BEIB, DES
R. Bonner	Biophysicist	BEIB, DES
P. Smith	Laser Physicist	BEIB, DES

Objective

This is a Phase I Trial designed to determine the feasibility of treating patients with superficial bladder carcinoma with a combination of hematoporphyrin derivative (HpD) and laser light, and to judge tumor response.

Methods Employed

Eligible patients will receive hematoporphyrin derivative by intravenous injection. They will subsequently undergo a cystoscopy at which time light will be delivered to the involved portion of the bladder according to the clinical judgment of the treating physicians using an optical fiber coupled to an argon pumped dye laser which has been introduced into the bladder through the cystoscope. Following treatment, both cystoscopy and urine cytology will be regularly done to assess response to treatment. If partial responses are observed without serious side effects, repeat treatment will be performed. Patients who develop invasive bladder carcinoma will be taken off protocol and referred for appropriate treatment.

Major Findings

To date, no patients have been entered on to this protocol, although patients are expected to be treated in the near future. Appropriate light diffusing fibers which can be introduced into the cystoscope have been procured for treatment purposes. A specially designed cystoscope holder has been manufactured and described in a recent publication, which can be used for centering the optical fiber during photodynamic therapy at the time of cystoscopy.

Significance to Biomedical Research and the Program of the Institute

Photodynamic therapy represents a potentially useful mode of curative therapy for selected patients with superficial carcinoma of the bladder. If this is achievable without requiring that the patients have their urinary bladder removed, this will represent a major advance in treatment of superficial carcinoma of the bladder.

Proposed Course

We propose to begin accruing patients for photodynamic therapy in the next few months. Twelve patients are currently approved for study on the protocol.

Publication

1. Manyak MJ, Harrington FS, Glatstein E. A flexible cystoscope holder/ surgical retractor that really works, J Urol 1987;138:105-6.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06389-01 RO

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influence of Lung Density in Mantle Technique Chest Irradiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. van de Geijn	Radiation Physicist	ROB, NCI
Others:	A. Raubitschek	Radiotherapist	ROB, NCI
	R. Miller	Radiation Physicist	ROB, NCI
	J. Chen	Radiation Physicist	ROB, NCI
	Kathy Yeakel-Orr	Dosimetrist	ROB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3

PROFESSIONAL: 1

OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Radiation therapy aims at complete destruction of the proliferative capability of all clonogenic tumor cells, while not exceeding the tolerance for radiation damage to any of the tissues and organs. The biological effects depend on the amount and distribution of absorbed dose. Clinical dosimetry depends almost entirely on an underlying theoretical model. In chest irradiation, the field size, and shape dependent dose distribution is further determined by the size, and shape of the patient, as well as density distribution, and inside the irradiated volume. The need for accurate accounting for the latter is still not unequivocally recognized. The present project is designed to shed new light on this problem.

The dosimetry of a large number of patients will be investigated using computer generated dosimetry, as well as relevant measurements.

Project Description

Objectives: To determine the influence of the presence of inhomogeneous tissues in irregularly shaped fields in the treatment of Hodgkin's disease in the chest.

Methods Employed

1. Generation of dose distributions in multiple sections as many patients as deemed necessary, with and without correction for lung density; CT-backed documentation is available on over 100 patients.

2. Generation of Dose-Volume histograms for both conditions.

3. Performance of adequate supporting measurements in suitable phantom conditions.

4. Two or more different density correction methods will be compared.

Major Findings

Although many computerized treatment planning systems are commercially or otherwise available, and accurate anatomical data can be obtained from CT, correction for lung density in calculated dose distributions is not common practice. Yet, in order to properly evaluate clinical results, it would seem that arguments either way would be supported by systematic investigative results.

Significance to Biomedical Research and the Program of the Institute

Evaluation of results of radiation treatment regimens require accurate dosimetry data on individual patients. Depending on the outcome of the proposed work, guidelines might emerge to facilitate more meaningful use of data from inter-institutional studies.

Proposed Course.

1. Available patient material will be used to simulate dose distributions for an adequate spectrum of cases, using different correction methods in common usage, in addition to one locally developed method.

2. Suitable phantom measurements will be performed.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 03800-18 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Surgical Consultants & Collaborative Research Involving Surgical Services at NIH

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.A. Rosenberg	Chief of Surgery, NCI	SURG, NCI
Others:	Entire Staff	Surgery Branch	SURG, NCI

COOPERATING UNITS *(if any)*
GD Aurbach (NIAMDD), JL Doppman (CC), E Glatstein (NCI), J Robbins (NIAMDD), L Liotta (NCI), RC Young (NCI), P Pizzo (NCI), J Gardner (NIAMDD)

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

Over 1000 consultations were received last year from other NCI Branches as well as other NIH Institutes.

SURGICAL SERVICES DEPARTMENT

ANNUAL STATISTICS

APRIL 1987 - MARCH 1988

TOTAL PROCEDURES	HOURS	INSTITUTES/OTHERS
426	1201.25	Ward (NCI)
948	1958.75	Consult (NCI)
95	164.75	Med. Br. (NCI)
1469	3324.75	TOTAL (NCI)
1469	3324.75	NCI
321-1/2	1362.75	NHLBI
164	732.50	NINCDS
61	61.25	Med. Neuro
73-1/2	140.75	NEI
38-1/2	78.00	ENT
		ROB
17	66.75	NIDR
9-1/2	20.50	Orthopedics
32	61.25	NICHD
1	.75	Other - (Anes)

TOTAL PROCEDURES	
123	Emergencies
268	Add-ons
522	Cancellations
346	OPD's
26	2WCSR
2	ICU-2J
5	MICU-10D
8	Other
5	Radiation
2187	Total Cases
5849.25	Total Hours

MONTHLY SUMMARY

January	176	Total Procedures	July	211	Total Procedures
	478	Total Hours		514.25	Total Hours
February	190	Total Procedures	August	173	Total Procedures
	568.50	Total Hours		453.25	Total Hours
March	224	Total Procedures	September	163	Total Procedures
	611.25	Total Hours		482.75	Total Hours
April	188	Total Procedures	October	175	Total Procedures
	499.50	Total Hours		431.25	Total Hours
May	163	Total Procedures	November	162	Total Procedures
	458.50	Total Hours		415.75	Total Hours
June	178	Total Procedures	December	184	Total Procedures
	456.25	Total Hours		480	Total Hours

1. Kern KA, Shawker TH, Doppman JLk, Miller DL, Marx SJ, Spiegel AM, Aurbach GD, Norton JA. The use of high-resolution ultrasound to locate parathyroid tumors during reoperations for primary hyperparathyroidism, World Journal of Surg 1987;11:579-85.

2. Cromack DT, Norton JA, Sigel B, Shawker TH, Doppman JL, Maton PN, Jensen RT. The use of high-resolution intraoperative ultrasound to localize gastrinomas: An initial report of a prospective study, World Journal of Surg 1987;11:648-53.

3. Skibber JM, Matter GJ, Pizzo PA, Lotze MT. Right lower quadrant pain in young patients with leukemia. A surgical perspective, Annals of Surgery 1987;206:711-6.

4. Norton JA, Peacock JL, Morrison SD. Cancer cachexia critical reviews, Oncology/Hematology 1987;38:18-20.

5. Norton JA, Cornelius MJ, Doppman JL, Maton PN, Gardner JD, Jensen RT. Effect of parathyroidectomy in patients with hyperparathyroidism, Zollinger-Ellison Syndrome, and multiple endocrine neoplasia type I: A prospective study, Surgery 1987;102:958-66.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03801-18 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Studies in Cancer Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: S.A. Rosenberg Chief of Surgery, NCI SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant diseases. The major emphasis of these studies is in the treatment of soft tissue sarcomas, osteogenic sarcomas, colorectal cancer, gastric cancer, renal cell cancer and melanoma. The major emphasis in Surgery Branch cancer therapy is in adjuvant therapy with emphasis on the use of combined treatment modalities in addition to surgery.

PUBLICATIONS

1. Inculet RI, Stein TP, Peacock JL, Leskiw M, Maher M, Gorschboth CM, Norton JA. Altered leucine metabolism in noncachectic sarcoma patients, Cancer Research 1987;47:4746-9.

2. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone, New Eng J of Med 1987;316:889-97.

3. Wiebke EA, McDonald HD, Pass HI. The infected chest wall prosthesis: Management by excision and contralateral transverse rectus abdominis musculocutaneous flap, Annals of Thor Surg 1987;44:544-5.

4. Chang AE, Schneider PD, Sugarbaker PH, Simpson C, Culnane M, Steinberg SM. A prospective randomized trial of regional versus systemic continuous 5-fluorodeoxyuridine chemotherapy in the treatment of colorectal liver metastases, Ann of Surg 1987;206:685-93.

5. Topalian SL, Rosenberg SA. Tumor-specific lysis by long-term cultured tumor-infiltrating lymphocytes. Surg Forum 1987;38:434-5.

6. Peacock JL, Inculet RI, Corsey R, Ford DB, Rumble WF, Lawson D, Norton JA. Resting energy expenditure and body cell mass alterations in noncachectic patients with sarcoma. Surg 1987;102:465-72.

7. Hoekstra HJ, Sindelar WF, Kinsella TJ, Oldhoff J. History, preliminary results, complications, and future prospects of intraoperative radiotherapy, Journal of Surg Onc 1987;36:175-82.

8. Lotze MT. Surgical management of hepatocellular carcinoma, Gastroenterology Clinics of North America 1987;16:613-26.

9. Topalian SL, Rosenberg SA. The therapy of cancer using the adoptive transfer of activated killer cells and interleukin-2, Acta Haematol 1987; 78:75-6.

10. Topalian SL, Solomon D, Avis FP, Chang AE, Freerksen DL, Linehan WM, Rosenberg SA. Immunotherapy of patients with advanced cancer using tumor infiltrating lymphocytes and recombinant interleukin-2: A pilot study, J Clin Oncol 1988;20:326-31.

11. Aebersold P, Carter CS, Hyatt C, Johnson S, Ottaway K, Leitman SF, Rosenberg, S.A. A simplified automated procedure for generation of human lymphokine activated killer cells for use in clinical trials, J Immunol Meth; in press.

12. Rosenberg SA, Lotze MT, Mulé JJ. New approaches to the immunotherapy of cancer using interleukin-2, Ann Int Med; in press.

13. Rizzoni WE, Miller K, Rick M, Lotze MT. Heparin-induced thrombocytopenia and thromboembolism in the postoperative period, Surgery; in press.

14. Simpson C, Seipp CA, Rosenberg SA. The current status and future applications of interleukin-2 and adoptive immunotherapy in cancer treatment, Sem in Oncol; in press.

15. Pogrebniak HW, Stovroff M, Roth JA, Pass HI. Resection of pulmonary metastases from malignant melanoma: Results of Pulmonary Metastases from Malignant Melanoma: Results of a 16-Year Experience, Ann of Thor Surg; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03811-14 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
The Immunotherapy of Animal and Human Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: S. A. Rosenberg Chief of Surgery, NCI SURG, NCI
Others: J. McIntosh (Medical Staff Fellow), J. Mulé (Senior Staff Fellow), S. Topalian (Senior Staff Fellow), B. Fox (Senior Staff Fellow), B. Packard (Staff Fellow), R. Puri (Senior Staff Fellow), A. Belldegrun (Visiting Scientist), A. Eisenthal (Visiting Associate), P. Aebersold (Expert), R. Cameron (Medical Staff Fellow), A. Kasid (Senior Staff Fellow), J. Weber (Senior Staff Fellow)

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
14	8	6

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Attempts are being made to develop new immunotherapeutic techniques for the treatment of advanced cancer. A variety of animal models are being used to test the effects of lymphokine activated killer cells, tumor infiltrating lymphocytes and combinations of lymphokines including interleukin-2, tumor necrosis factor and alpha-interferon in the treatment of experimental animal tumors. Current research is attempting to define the factors necessary for achieving successful adoptive immunotherapy in experimental animal models.

A variety of clinical trials are also in progress exploring the application of new adoptive immunotherapies to patients with advanced cancer. Clinical trials are exploring the value of lymphokine activated killer cells and interleukin-2, high-dose interleukin-2 alone, the combination of alpha-interferon and interleukin-2, the combination of interleukin-2 and tumor necrosis factor, and the value of colony stimulating factors in cancer treatment.

Newer efforts are directed at transducing new genes into tumor infiltrating lymphocytes that can increase their therapeutic effectiveness.

PUBLICATIONS

1. Vetto JT, Papa MZ, Lotze MT, Chang AE, Rosenberg SA. Reduction of toxicity of interleukin-2 and lymphokine activated killer cells in humans by the administration of corticosteroids, J Clin Oncol 1987;5:496-503.

2. Ettinghausen SE, Rosenberg SA. Vascular leak syndrome (VLS) associated with the administration of lymphokine-activated killer cells (LAK) and interleukin-2 (IL-2) to mice, Surg Forum 1987;37:388-9.

3. Shiloni E, Eisenthal A, Sachs D, Rosenberg SA. Antibody dependent cellular cytotoxicity mediated by murine lymphocytes activated in recombinant interleukin-2, J Immunol 1987;138:1992-8.

4. Asher A, Mulé JJ, Reichert CM, Shiloni E, Rosenberg SA. Studies on the anti-tumor efficacy of systemically administered recombinant tumor necrosis factor against several murine tumors *in vivo*, J Immunol 1987;138:963-74.

5. Ottow RT, Steller EP, Sugarbaker PH, Wesley RA, Rosenberg SA. Immunotherapy of intraperitoneal cancer with interleukin-2 and lymphokine activated killer cells reduces tumor load and prolongs survival in murine models, Cell Immunol 1987;104:366-76.

6. Muul LM, Spiess PJ, Director EP, Rosenberg SA. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma, J Immunol 1987;138:989-95.

7. Chang AE, Matory YL, Dwyer AJ, Hill SC, Girton ME, Girton RT, Steinberg SM, Knop RH, Frank JA, Hyams D, Doppman JL, Rosenberg SA. Magnetic resonance imaging versus computed tomography in the evaluation of soft tissue tumors of the extremities, Ann Surg 1987;205:340-8.

8. Fauci AS, Rosenberg SA, Sherwin SA, Dinarello CA, Longo DL, Lane HC. Immunomodulators in clinical medicine, Ann Int Med 1987;106:421-33.

9. Mulé JJ, Rosenberg SA. Successful adoptive immunotherapy of established metastases with lymphokine activated killer cells and recombinant interleukin-2. In Herberman RB, Wiltrout RH, Gorelik E, eds. Immune Responses to Metastases. Florida: CRC Press, Inc., 1987; 69-94.

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11. Ettinghausen SE, Moore JG, White DE, Platanias L, Young NS, Rosenberg SA. Hematologic effects of immuntherapy with lymphokine-activated killer cells and recombinant interleukin-2 in cancer patients, Blood 1987;69:1654-60.

12. Lotze MT, Custer MC, Sharrow SO, Rubin LA, Nelson DL, Leonard WJ, Rosenberg SA. In vivo administration of purified human interleukin-2. Development of interleukin-2 receptor positive cells and circulating soluble IL-2 receptors following IL-2 administration to humans, Cancer Res 1987; 47:2188-95.

13. Shu S, Chou T, Rosenberg SA. Generation from tumor-bearing mice of lymphocytes with *in vivo* therapeutic efficacy, J Immunol 1987;139:295-304.

14. Eisenthal A, Lafreniere R, Lefor AT, Rosenberg SA. The effect of anti B16 melanoma monoclonal antibody on established murine B16 melanoma liver metastases, Cancer Res 1987;47:2771-76.

15. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE. A progress report on the treatment of 157 patients with advanced cancer using lymphokine activated killer cells and interleukin-2 or high dose interleukin-2 alone, N Engl J Med 1987;316:889-905.

16. Belldegrun A, Webb DE, Austin HA, Steinberg SM, White DE, Linehan WM, Rosenberg SA. Effects of interleukin-2 on renal function in patients receiving immunotherapy for advanced cancer, Ann Int Med 1987;106:817-22.

17. Mulé JJ, Yang JC, Lafreniere R, Shu S, Rosenberg SA. Identification of cellular mechanisms operational in vivo during the regression of established pulmonary metastases by the systemic administration of high-dose recombinant interleukin-2, J Immunol 1987;139:285-94.

18. Lotze MT, Rosenberg SA. Letter to the Editor, JAMA 1987;157:1730-31.

19. Topalian SL, Muul LM, Solomon D, Rosenberg SA. Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials, J Immunol Meth 1987;102:127-41.

20. Skibber JM, Lotze MT, Uppenkamp I, Ross W, Rosenberg SA. Identification and expansion of human lymphokine activated killer cells: Implications for the immunotherapy of cancer, J Surg Res 1987;42:613-21.

21. Mulé JJ, Smith CA, Rosenberg SA. Interleukin-4 (B-cell stimulatory factor-1) can mediate the induction of LAK activity directed against fresh tumor cells, J Exp Med 1987;166:792-7.

22. Denicoff KD, Rubinow DR, Papa MZ, Simpson C, Seipp CA, Lotze MT, Chang AE, Rosenstein D, Rosenberg SA. The neuropsychiatric effects of interleukin-2/lymphokine activated killer cell treatment, Ann Int Med 1987;107:293-300.

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24. Muul LM, Nason-Burchenal K, Carter CS, Cullis H, Slavin D, Hyatt C, Director EP, Leitman SF, Klein HG, Rosenberg SA. Development of an automated closed system for generation of human lymphokine activated killer (LAK) cells for use in adoptive immunotherapy, J Immunol Meth 1987;101: 171-81.

25. Roberts K, Lotze MT, Rosenberg SA. Separation and functional studies of the human lymphokine activated killer cell, Cancer Res 1987;47:4366-71.

26. Ward BA, Chou T, Chang AE, Shu S, Rosenberg SA. Specific adoptive immunotherapy: Characterization of lymphoid subpopulations involved in tumor rejection, Surg Forum 1987;37:416-8.

27. Topalian SL, Rosenberg SA. Therapy of cancer using the adoptive transfer of activated killer cells and interleukin-2, Acta Haematologica 1987;78:1-2.

28. Rosenberg SA. Letter to the Editor, N Engl J Med 1987;317:962-3.

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30. Mulé JJ, Rosenberg SA. Mechanistic aspects of successful immunotherapy of established pulmonary metastases by the systemic administration of high-dose recombinant interleukin-2. In: Cellular Immunotherapy of Cancer. Alan R. Liss, Inc., 1987;79-91.

31. Muul LM, Burchenal K, Hyatt C, Schwarz S, Slavin D, Director E, Rosenberg SA. Studies of serum free culture medium in the generation of lymphokine activated killer cells, J Immunol Meth 1987;105:183-92.

32. Topalian SL, Rosenberg SA. Tumor-specific lysis by long-term cultured tumor infiltrating lymphocytes, Surg Forum 1987;38:434-57.

33. Cohen PJ, Lotze MT, Roberts JR, Rosenberg SA, Jaffe ES. The immunopathology of sequential tumor biopsies in patients treated with interleukin-2. Correlation of response with T-cell infiltration and HLA-DR expression, Am J Path 1987;129:208-16.

34. Weber JS, Jay G, Rosenberg SA. Immunotherapy of a murine tumor with interleukin-2; increased sensitivity after MHC Class I gene transfection, J Exp Med 1987;166:1716-33.

35. Wiebke EA, Lotze MT, Rosenberg SA. Tumor cell susceptibility to lysis: Marked increase in lysis by tumor infiltrating lymphocytes following target stimulation with interferon- and tumor necrosis factor- . Implications for immunotherapy, Surg Forum 1987;38:436-8.

36. Spiess PJ, Yang JC, Rosenberg SA. In vivo antitumor activity of tumor infiltrating lymphocytes expanded in recombinant interleukin-2, J Natl Cancer Inst 1987;79:1067-75.

37. Chang AE, Rosenberg SA. Immunotherapy of cancer utilizing interleukin-2 and lymphokine activated killer cells. In: Kimura K, et al., eds. Cancer Chemotherapy: Challenges for the Future. 1987;2:38-45.

38. Belldegrun A, Topalian SL, Linehan M, Robertson C, Rosenberg SA. Growth and Antitumor activity of tumor-involved draining lymph node cells versus tumor-infiltrating lymphocytes from patients with renal cell cancer, Surgical Forum 1987;38:688-90.

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40. Rosenberg SA. The development of new immunologic approaches to cancer therapy, Blood Purification 1988;6:69-76.

41. Belldegrun A, Uppenkamp I, Rosenberg SA. Anti-tumor reactivity of human lymphokine activated killer (LAK) cells against fresh and cultured preparations of renal cell cancer, J Urol 1988;139:150-5.

42. Papa MZ, Yang JC, Vetto JT, Shiloni E, Eisenthal A, Rosenberg SA. Combined effects of chemotherapy and interleukin-2 in the therapy of mice with advanced pulmonary tumors, Cancer Res 1988;48:122-9.

43. Rosenberg SA. Immunotherapy of patients with advanced cancer using interleukin-2 alone or in combination with lymphokine activated killer cells. In: DeVita V, Hellman S, Rosenberg SA, eds. Important Advances in Oncology. Philadelphia: J B Lippincott Co, 1988; 217-57.

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45. Gaspari AA, Lotze MT, Rosenberg SA, Stern JB, Katz SI. Dermatologic changes associated with interleukin-2 administration, JAMA 1988; in press.

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47. Lotze MT, Rosenberg SA. Interleukin-2 as a pharmacologic reagent. In: K Smith, ed. Lymphokines. Florida: Academic Press, Inc., 1988; in press.

48. Yang JC, Mulé JJ, Rosenberg SA. In vivo requirement for Asialo GM1-bearing cells in the treatment of murine sarcoma with lymphokine activated killer cells with therapeutic efficacy, Intl J Cancer 1988; in press.

49. Eisenthal A, Shiloni E, Rosenberg SA. Characterization of IL-2 induced murine cells which exhibit ADCC activity, Cellular Immunol 1988; in press.

PUBLICATIONS

50. Saris SC, Rosenberg SA, Friedman RB, Rubin JT, Barba D, Oldfield EH. Penetration of recombinant interleukin-2 across the blood-cerebrospinal fluid barrier, J Neurosurgery 1988; in press.

51. Eisenthal A, Rosenberg SA. Cross linking of anti-B16 melanoma monoclonal antibodies to lymphokine activated killer (LAK) cells, Intl J Cancer 1988; in press.

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53. Chang AE, Kinsella T, Glatstein E, Baker AR, Sindelar WF, Lotze MT, Danforth DN, Sugarbaker PH, Lack EE, Steinberg SM, White DE, Rosenberg SA. Adjuvant chemotherapy for patients with high grade soft tissue sarcomas of the extremity, J Clin Oncol 1988; in press.

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55. Fox BA, Rosenberg SA. Development of a monoclonal antibody which separates heterogeneous lymphokine activated killer cell presursor populations, J Immunol 1988; in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06654-11 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies in Malignant Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I. W. F. Sindelar Senior Investigator SURG, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with gastrointestinal carcinomas have been studied for evidence of reactivity against tumor-associated determinants expressed on both fresh and cultured syngeneic or allogeneic tumor cells using immunoperoxidase staining techniques. Tumor-associated antigens have been isolated from both animal and human pancreatic cancers and have been investigated for possible applications to immunotherapy or methods of immunodiagnosis. Monoclonal antibodies have been developed to tumor-associated determinants in both hamster and human pancreatic cancers and have been demonstrated to be cytotoxic cells both in vitro and in vivo. Tolerance of various normal and surgically-manipulated tissues to intraoperative radiotherapy has been investigated in dogs to determine both acute and long-term toxicity from radiation effects. Clinical trials of intraoperative radiotherapy have been performed including feasibility and developmental studies, randomized trials in resectable and unresectable pancreatic carcinoma, randomized trials in gastric carcinoma, and randomized trials in retroperitoneal sarcomas.

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2. Garra BS, Shawker TH, Doppman JL, Sindelar WF. Comparison of angiography and ultrasound in the evaluation of the portal venous system in pancreatic carcinoma, J Clin Ultrasound 1987;15:83-93.

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13. Hoekstra HJ, Restrepo C, Kinsella TJ, Sindelar WF. Histopathological effects of intraoperative radiotherapy on pancreas and adjacent tissues: A postmortem analysis, J Surg Oncol 1988;37:104-8.

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15. Kinsella TJ, Sindelar WF, Lack E, Glatstein E, Rosenberg SA. Preliminary results of a randomized study of adjuvant radiation therapy in resectable adult retroperitoneal soft tissue sarcomas, J Clin Oncol 1988;6:18-25.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06657-06 SURG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies with TNF and Insulin in Cancer Cachexia and Zollinger-Ellison Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J. A. Norton	Senior Investigator	SURG, NCI
Others:	D. Cromack	Medical Staff Fellow	SURG, NCI
	D. Fraker	Medical Staff Fellow	SURG, NCI
	M. Stovroff	Medical Staff Fellow	SURG, NCI
	B. Sheppard	Medical Staff Fellow	SURG, NCI
	G. Darling	Medical Staff Fellow	SURG, NCI
	C. Gorschboth	Medical Technologist	SURG, NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TNF decreases food intake and body weight gain in rats, but with repeated i.p. administration rats become tolerant to the effects of TNF on food intake and body weight gain.[1] When tolerant rats are given a subsequent inoculation of sarcoma, they live significantly longer than control non-tolerant rats.[1] Rats tolerant to TNF's effects on food intake and body weight are also tolerant to a lethal injection of TNF or endotoxin(LPS).[2] Tolerance to LPS also protects against TNF.[2] However, the mechanisms of tolerance to LPS and TNF are not the same, because LPS tolerance decreases macrophage secretion of TNF and TNF tolerance increases macrophage secretion of TNF.[3]

Sarcoma-bearing rats have circulating cachectin activity in their serum and levels increase as food intake decreases, body weight decreases and tumor burden increases.[4] Tumor resection reverses serum cachectin activity levels.[4] This experiment[4] plus the previous one[1] suggest that TNF is a mediator of cachexia in this rat model. This sarcoma has high-affinity glucocorticoid receptors which regulate its growth.[5] Insulin is able to reverse the toxic effects of TNF including effects on body weight, food intake and histology.[6] Insulin is also able to improve the body composition of sarcoma-bearing rats preserving lean body and fat mass,[7] and in some experiments insulin can improve the survival of cachectin TB rats.[8]

Gastrinomas in patients with ZES can be occult and difficult to find at surgery. We prospectively compared operative ultrasound to palpation to localize gastrinomas and found that each procedure complements each other.[9] In addition, we demonstrated more effective management of gastric acid hypersecretion by parathyroidectomy in patients with ZES, hyperparathyroidism and MEN-I.[10] Finally, we demonstrated a 40% objective response rate of combination chemotherapy including streptozotocin, 5 FU and doxorubicin in patients with metastatic gastrinoma.[11]

PUBLICATIONS

1. Fraker DL, Norton JA. Reversal of the toxic effects of cachectin by insulin, Surgical Forum 1987;38:50-2.

2. Norton JA, Cornelius MJ, Doppman JL, et al. Effect of parathyroidectomy in patients with hyperparathyroidism and Zollinger-Ellison syndrome and multiple endocrine neoplasia type I: a prospective study, Surgery 1987;102: 1018-26.

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4. Moley JF, Morrison SD, Gorschboth CM, Norton JA. Body compositional changes in rats with experimental cancer cachexia: improvement with exogenous insulin, Cancer Res 1988;48:2784-7.

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6. Norton JA, Cromack DT, Shawker TH, et al. Intraoperative ultrasonographic localization of islet cell tumors: a prospective comparison to palpation, Ann Surg 1988;207:160-8.

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9. Fraker DL, Stovroff MC, Marino M, Norton JA. Tolerance to tumor necrosis factor in rats and the relationship to endotoxin tolerance and toxicity, J Exp Med 1988; in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06658-06 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Melatonin/Estrogen Receptor Structure in Human Breast Cancer Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.	D. N. Danforth, Jr.	Senior Investigator	SURG, NCI
Others:	B. Gabriel	Staff Fellow	SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We are studying the effect of melatonin on protein and RNA metabolism in human breast cancer cells. We have found that melatonin rapidly (within 15 minutes) enhances protein synthesis. Enhancement is self limiting and complete within 40 minutes. Analysis of subcellular fractions at 40 minutes indicates that the protein content of mitochondria, microsomes, and cytosol significantly increased. Pulse chase studies at 3 hours demonstrate significant increase in nuclear content of the induced protein. Gel electrophoresis of cytosol from melatonin-treated cells indicates that one or more polypeptides in the 3000 molecular weight range are induced by melatonin. Melatonin also stimulates total cellular RNA synthesis, beginning at 6-12 hours. This stimulation is blocked by cycloheximide and therefore requires the prior action of melatonin on protein synthesis. In vitro studies of nucelar DNA-dependent RNA polymerase indicate that melatonin enhances both polymerase I and II, and therefore stimulates both ribosomal and messenger RNA synthesis. Analysis of ^{3}H-uridine labeled ribsomal RNA by agarose gel electrophoresis indicate that both 18S & 28S RNA are being stimulated. Studies of the in vitro translation products of melatonin stimulated m-RNA suggest that the transcription of message for multiple small polypepitides is being stimulated by melatonin. Studies to further characterize these polypeptides are in progress. We have also used the covalently attaching label ^{3}H-tamoxifen aziridine to study the molecular weight forms of the estrogen receptor in human breast cancer cells. By denaturing gel electrophoresis the estrogen receptor migrates as the subunit protein (67K form.) Under low salt conditions the subunit form aggregates to larger proteins (240K form), which are readily dissociated by the addition of salt. Analysis in the presence of protease inhibitors indicate the presence of two discrete large molecular weight forms which are not dissociated by salt. These forms are felt to be large molecular weight precursors for the estrogen receptor subunit. These methods are now being used to characterize estrogen receptors protein in human breast cancer tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06659-06 SURG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*

Studies of Urologic Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	W. M. Linehan	Head, Urologic Oncology Section	SURG, NCI
Others:	C. N. Robertson	Senior Investigator	SURG, NCI
	Leonard L. Gomella	Medical Staff Fellow	SURG, NCI
	Terence P. Wade	Medical Staff Fellow	SURG, NCI
	Patrick Anglard	Visiting Fellow	SURG, NCI
	Eric Sargent	Guest Researcher	SURG, NCI
	Elizabeth Miller	Microbiologist	SURG, NCI
	Emile Trahan	Medical Technician	SURG, NCI

COOPERATING UNITS *(if any)*

NCI (Berton Zbar); NCI (Attan Kasid)

LAB/BRANCH

Surgery Branch

SECTION

Urologic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.5	5	1.5

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

We are studying the molecular genetics of renal cell carcinoma, evaluating growth factor production by genitourinary tumors and participating in studies of adoptive immunotherapy in patients with advanced malignancies. By use of the molecular technique of restriction fragment length polymorphism analysis we have identified DNA sequence deletions in the short arm of chromosome 3 in sporadic renal cell carcinoma. The loss of alleles at loci on the short arm fo chromosome 3 may indicate the presence of a renal carcinoma recessive oncogene at this location. We have also identified DNA sequence deletions at chromosome 11 and also at chromosome 13 at the retinoblastoma locus. We have demonstrated, also in collaboration with Berton Zbar, DNA sequence deletions in the short arm of chromosome 3 in kidney tumors and pheochromocytoma from patients with a familial form of renal cell carcinoma, Von Hippel-Lindau syndrome. We have demonstrated that chromosome 3s retained in the renal tumors of the proband were inherited from the affected parent which is consistent with our previous data suggesting that the RCC gene is located on chromosome 3p and that 3p allele loss represents the second step of a two-mutation process. DNA sequence deletions observed at other chromosomal loci (11 and/or 13) may be important in progression or metastasis. We have demonstrated loss of heterozygosity at loci on chromosome 11 and 17 in a related tumor, adrenocortical carcinoma. In study of the effect of tumor produced growth factors we have demonstrated by Northern blot analysis that human renal cell carcinoma has an increased expression of the transforming growth factors TGF-alpha and TGF-beta and that the tumor produces TGF-beta in a biologically inactive form. We have shown that TGF-beta inhibits the growth of renal cell carcinoma. These studies may provide a basis for the development of new therapeutic strategies for treatment of this malignancy as well as increase our understanding of how tumor produced factors affect immunologic response. We have evaluated patients with metastatic and locally advanced renal cell carcinoma as part of this protocol and in support of basic and clinical studies of tumor infiltrating lymphocytes.

1. Brauch H, Johnson B, Hovis J, Yano T, Graziano S, Sorenson GD, Poiesz BJ, Minna J, Linehan M, Zbar B. Molecular analysis of the short arm of chromosome 3 in small cell and non-small cell carcinoma of the lung, N Engl J Med 1987;317:1109-13.

2. Andriole GL, Sandlund JT, Miser JS, Arasi V, Linehan M, Magrath IT. The efficacy of mesna (2-mercaptoethane sodium sulfonate) as a uroprotectant in patients with hemorrhagic cystitis receiving further oxazaphosphorine chemotherapy, J Clin Oncol 1987;5:799-803.

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4. Topalian SL, Solomon D, Avis F, Chang AE, Freerksen DL, Linehan WM, Lotze MT, Robertson CN, Seipp CA, Simon P, Simpson CG, Rosenberg SA. Immunotherapy of patients with advanced cancer using tumor-infiltrating lymphocytes and recombinant interleukin-2: A pilot study, J Clin Oncology 1988; 6:839-53.

5. Whitcomb RW, Linehan WM, Wall LM, Knazek RA. Monocytes stimulate cortisol production by cultured human adrenocortical cells, J Clin Endocrinol Metab 1988;66:33-8.

6. Linehan WM. Renal cell carcinoma (editorial), J Urol 1988;139:340-1.

7. Whitcomb RW, Linehan WM, Knazek RA. Effects of long-chain, saturated fatty acids on membrane microviscosity and adrenocorticotropin responsiveness of human adrenocortical cells in vitro, J Clin Invest 1988;81:185-8.

8. Ozols R, Ihde D, Linehan WM, Jacob J, Ostchega Y, Young RC. A randomized trial of standard chemotherapy versus a high dose chemotherapy regimen in the treatment of poor prognosis nonseminomatous germ-cell tumors, J Clin Oncology 1988; in press.

9. Webb DE, Austin HA, Belldegrun A, Vaughan E, Linehan WM, Rosenberg SA. Metabolic and renal effects of interleukin-2 immunotherapy for metastatic cancer, Clin Nephrology 1988; in press.

10. Linehan WM, Shipley W, Longo D. Cancer of the kidney and ureter. In: DeVita VT, Hellman S, Rosenberg SA, eds. Principles and Practices of Oncology. Philadelphia: J. B. Lippincott, 1988; in press.

11. Solomon D, Merino M, Linehan WM, Jaffe E. Angiocentric malignant lymphoma mimicking renal cell carcinoma in fine needle aspiration cytology, Surgical Pathology 1988; in press.

12. Gomella LG, Sargent ER, Anglard P, Linehan WM, Kasid A. Overexpression of the epidermal growth factor gene in human renal cell carcinoma tissues, Surgical Forum 1988; in press.

13. Linehan WM, Robertson CN, Rosenberg SA. Clinical experience with adoptive immunotherapy in patients with renal cell carcinoma, Urology Annual 1988; in press.

14. Gomella L, Linehan WM. Bladder cancer, new and potential approaches. In: Magrath ET, ed. New and Potential Approaches to Cancer Therapy. Springer Verlag 1988; in press.

15. Linehan WM, Robertson CN, Miller ET, Santora AC. Evaluation of the paracrine and endocrine effects of genitourinary produced growth factors. In: Javadpour N, ed. Advances in Urology. New York: Alan R. Liss, Inc. 1988; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06660-05 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Study of Interleukin-2 Based Immunotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. C. Yang	Senior Investigator	SURG, NCI
Others:	D. Perry-Lalley	Microbiologist	SURG, NCI
	K. Griffith	Medical Staff Fellow	SURG, NCI
	E. Shlasko	Medical Staff Fellow	SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	3.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory is studying ways to generate, expand, and therapeutically administer lymphoid cells with antitumor effects to tumor-bearing animals. We are currently examining in situ tumor immunity and its exploitation using interleukin-2 to culture tumor-infiltrating lymphocytes (TIL). Our approach is to first optimize in situ immunity through studies of tumor immunogenicity, host response to transplanted murine tumors and the effects of exogenous lymphokine therapy. Secondly, techniques for the in vitro culture of TILs maintaining maximal therapeutic efficacy will be investigated. These techniques include manipulations of growth factors, feeder cells, and tumor antigen. Thirdly, investigations on the in vivo traffic of transferred cells will continue with the objective of clarifying and enhancing the conditions which cause localization of cells to tumor sites. Lastly, in vitro parameters which can predict therapeutic efficacy of TILs are being sought so that rational treatment strategies can be designed based on these predictors of response.

A separate project which is on-going is to investigate the mediators of toxicity during interleukin-2 therapy. Studies will include mainly investigation of leukotriene levels and inhibitors of the formation of leukotrienes. In addition, cell transfer experiments investigating the cellular mediators of toxicity are on-going.

1. Spiess PJ, Yang JC, Rosenberg SA. In vivo antitumor activity of tumor-infiltrating lymphocytes expanded in recombinant interleukin-2, JNCI 1987;5:79.

2. Papa MZ, Yang JC, Vetto JT, Shiloni E, Eisenthal A, Rosenberg SA. Combined effects of chemotherapy and interleukin 2 in the therapy of mice with advanced pulmonary tumors, Cancer Res 1988;48:122-29.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06661-05 SURG

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Immunologic Studies in Patients with Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.T. Lotze	Senior Investigator	SURG, NCI
Others:	M.C. Custer	Microbiologist	SURG, NCI
	D. Jablons	Medical Staff Fellow	SURG, NCI
	J. Rubin	Medical Staff Fellow	SURG, NCI
	Y. Kawakami	Visiting Fellow	SURG, NCI
	H. Stotter	Visiting Associate	SURG, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
4.5	3.5	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory's major goal is the development and evaluation of immunologic reagents to treat patients with cancer. One goal has been to evaluate the in vivo effects of both IL-2 and TNF and to determine the mechanism of anti-tumor effects as well as induction of toxicity. We have demonstrated that IL-2 has major effects on the endothelium, inducing endothelial activation antigens and believe that this may be associated with the capillary leak syndrome. In addition, we have demonstrated that IL-2 induces DR expression on many tumor cells as well as infiltrating T cells and that this appears to correlate with antitumor effects. In addition, IL-2 causes major endocrinologic effects, including the elaboration and release of ACTH, cortisol, and beta endorphin as well as CRH. Major effects on the traffic of peripheral blood mononuclear cells are demonstrated with both IL-2 and TNF as is the elaboration of a novel cytokine, IL-6. Major decreases in the DTH response have been observed in patients undergoing IL-2 treatment. The second major goal of our laboratory is to evaluate the lymphokine-activated killer cell, both its generation as well as lytic specificity. We have demonstrated that normal human monocytes are susceptible to lysis and further that LAK cells can be generated from the bone marrow and peripheral blood with activity against variety of human tumors. Susceptibility to lysis by these effectors is modulated by interferon and TNF treatment of the target cells. Further, a variety of factors have been evaluated including IL-1, IL-3, IL-6, GMCSF, TNF and all of the interferons have been evaluated and found to have no major role in the generation or maintenance of LAK activity. IL-4 has profound effects regulating LAK activity and in addition, allows the enhanced generation of TILs from human melanoma. Evaluation of the role of GMCSF in enhancing poly function following in vivo administration has been evaluated. The role of murine monoclonal antibodies in mediating ADCC as well as their efficacy in in vivo studies in combination with IL-2 or other cytokines are in progress.

PUBLICATIONS

1. Cohen PJ, Lotze MT, Roberts JR, Rosenberg SA, Jaffe ES. The immunopathology of sequential tumor biopsies in patients treated with interleukin-2, Amer J of Path 1987;129:208-16.

2. Cotran RS, Pober JS, Gimbrone Jr MA, Springer TA, Wiebke EA, Gaspari AA, Rosenberg SA, Lotze MT. Endothelial activation during interleukin-2 immunotherapy. A possible mechanism for the vascular leak syndrome, J of Immunology 1987;139:1883-8.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06662-02 SURG

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Studies of Phototherapy for Thoracic Malignancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	H.I.Pass	Senior Investigator	SURG, NCI
Others:	W. Matthews	Medical Technician	SURG, NCI
	R. Perry	Medical Staff Fellow	SURG, NCI
	S. Evans	Medical Staff Fellow	SURG, NCI

COOPERATING UNITS *(if any)*

Radiation Oncology Branch, NCI (A. Russo, J. Mitchell)

LAB/BRANCH

Surgery Branch

SECTION

Thoracic Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER.
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our laboratory has continued investigations of photodynamic therapy (PDT) for the treatment of thoracic malignancies by sensitization of malignant cells with dihematoporphyrin ether (DHE) followed by illumination with 630 nM light. Since October of 1987 we have investigated the use of light scattering media, intralipid, in different concentrations to improve the efficacy of PDT. We have found that low concentrations of intralipid will provide greater cytotoxicity via PDT, with greater light-scattering effects despite a loss of the sensitizer from the cells during the period of incubation with the intralipid. Using a phantom plexiglass model of the thoracic cavity, we have defined what the optimal light scattering concentration in this model is for intralipid in order to provide equal illumination to all surfaces. We have quantitated light scattering using a photodiode detection system, constructed by the Bioengineering Division. We are now investigating different in vitro PDT sensitivities of various thoracic, oncologic cell lines including a small-cell lung cancer variant, a large-cell carcinoma of the lung, mesothelioma, normal lung fibroblasts and an adenocarcinoma. For each of these cell lines we are defining fluourescence characteristics of the cells, cell size, cell protein, and inherent sensitivity to low energy phototherapy. The role of LDL receptors in the delivery of DHE at a cellular level is also being investigated. In preparation for conjugation of a monoclonal antibody to the sensitizor, in vitro and in vivo studies of PDT of a transformed fibroblast line, 45342 have been performed showing sensitivity of this line to PDT both in vitro and in vivo.

We have now treated 9 patients with endobronchial disease with PDT and have made a movie demonstrating the technique of phototherapy. Two patients with recurrent ovarian carcinomatosis have been treated with intraabdominal PDT.

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BIOLOGICAL RESPONSE MODIFIERS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1987 to September 30, 1988

INTRODUCTION

The Biological Response Modifiers Program (BRMP) is unique within the National Cancer Institute (NCI) in that it is a comprehensive program with both extramural and intramural clinical research and basic research components charged with the investigation, development, and clinical testing of biological therapeutic agents that may alter host defenses or cancer cells directly in a fashion that results in tumor eradication. This unique structure facilitates the rapid application of basic science and preclinical observations into clinical settings.

The extramural activities of the BRMP are monitored and supervised by the Biological Resources Branch (BRB) under the direction of Dr. Stephen P. Creekmore. The program is composed of a balance of grants and contracts supporting preclinical and clinical research throughout the biomedical research community. The BRB sponsors Phase I and Phase II clinical trials of biological agents looking not only for antitumor effects but also for modifications in selected immune or biological functions that could be associated with an antitumor response. The BRB maintains a close working relationship with the Developmental Therapeutics Program (DTP) through the BRMP/DTP Working Group (BDWG) to coordinate preclinical development of biological agents with our sister program in preclinical drug development; and with the Cancer Therapy Evaluation Program (CTEP) through the BRMP/CTEP Working Group (BCWG) to coordinate the Phase III clinical development of biological agents with our sister program in clinical drug development. The BRB supervises an intramural contract-supported preclinical evaluation laboratory that evaluates biological agents in preclinical models that may have specific impact on the design and monitoring of human clinical trials, including the study of dose- and schedule-related variables and the biological and immunological parameters associated with tumor regression in vivo. The BRB serves as liaison with a large number of biotechnology companies and studies mechanisms of interacting with commercial interests to foster biological therapeutic drug development. In addition, the BRB stimulates therapy-directed research through the sponsorship of scientific meetings, the establishment of standards for assessing the function of biological agents, and the announcement of RFAs on particularly significant research topics.

The intramural program of the BRMP consists of three basic science laboratories and one clinical branch: the Laboratory of Molecular Immunoregulation (LMI), the Laboratory of Experimental Immunology (LEI), the Laboratory of Biochemical Physiology (LBP), and the Clinical Research Branch (CRB). The LMI consists of two sections: the Immunobiology Section (IS) headed by Dr. Luigi Varesio and the Lymphokines Section (LS) under Dr. Frank Ruscetti. The LMI laboratory chief is Dr. Joost Oppenheim. The LMI (1) investigates at a molecular level the intercellular and intracellular signals that regulate host defense mechanisms; (2) studies the lymphokine/cytokine modulation of cellular functions in tumor cells and cells that participate in host defense; (3) devises new diagnostic tests to better define the immune status and pursue more critical evaluation of BRMs; (4) evaluates the effects of BRMs on tumor cells, immunoregulatory pathways, and host defense mechanisms; and (5) generates new BRMs that may modify tumor cells or host defense mechanisms.

The LEI is composed of three sections: the Cellular and Molecular Immunology Section (CMIS) under Dr. Craig Reynolds, the Leukocyte Differentiation Section (LDS) with Dr. John Ortaldo acting as interim head, and the Experimental Therapeutics Section (ETS) under Dr. Robert Wiltrout. The LEI laboratory chief is Dr. John Ortaldo. The LEI (1) conducts studies on BRMs and their application to the treatment of cancer; (2) studies the therapeutic use of cell-mediated immune effector mechanisms, lymphokines, monoclonal antibodies, growth factors and their antagonists, chemotherapy and other components of the host response to cancer alone and in combination in experimental tumor models; (3) studies the cellular and molecular mechanisms by which effector cells mediate antitumor activity; (4) studies the control of macrophage and lymphocyte activation; and (5) prepares new monoclonal antibodies or modifies existing antibodies as therapeutic agents or as probes to analyze cell differentiation and function, especially lymphoid and hematopoietic cells.

The LBP laboratory chief is Dr. Hsiang-fu Kung. The LBP (1) plans and conducts research on the genetic and biochemical events related to the development and expression of malignant phenotypes in humans and animals; (2) applies skills in molecular biology, recombinant DNA technology and biochemistry to develop a comprehensive program to identify and isolate relevant genes and their products which relate to cell transformation, the control of cell growth and differentiation, and the regulation of host defenses; and (3) coordinates collaborative efforts between the BRMP and other intramural and extramural program elements in order to identify, clone, and isolate genes encoding molecules of potential value in the treatment of malignancy or the alteration of biological responses.

The CRB branch chief is Dr. Ronald Steis. The CRB (1) performs Phase I and Phase II clinical trials of selected biological therapeutic agents; (2) conducts innovative pilot studies integrating biologicals with other forms of treatment; (3) makes clinical correlations with extensive laboratory data collected to monitor the effects of biological agents on the host; and (4) supports the autologous bone marrow transplantation effort of the NIH Clinical Center, the DCT Clinical Oncology Program, and other NIH units in need of this service for immune reconstitution, intensive cancer chemotherapy or genetic therapy.

EXTRAMURAL PROGRAM ACCOMPLISHMENTS

There are extensive interactions with other programs with similar and potentially overlapping interests to facilitate smooth coordination of biological therapeutic drug development. As noted above, working groups with CTEP and DTP meet regularly to synergize and integrate activities. There is also a close working relationship with the Immunology and Tumor Biology programs of the Division of Cancer Biology and Diagnosis.

Grants Program

In FY88, there were 101 active grants with approximately $19 million in support. Of these, 74 were R01 grants, 10 were P01 program projects, 1 was an R35 (Outstanding Investigator Award), 3 were New Investigator Awards, 2 were R13 conference grants, 6 were merit awards, and 5 were Small Business Innovative Research (SBIR) grants. The major scientific areas supported by the BRMP grant program include: cytokines, monoclonal antibodies, enhanced drug delivery (e.g. utilizing liposomes), molecular biology as a tool for the study of oncogenes, and immunological studies of AIDS.

Requests for Application (RFAs)

In FY88, the BRMP issued three new RFAs: protection of bone marrow against the effects of cytotoxic drugs and X-irradiation; immunologic investigation of multidrug resistance of neoplastic cells; and manipulation of the suppressor arm of the immune response directed towards successful human immunotherapy. Four grants were funded from these solicitations. In addition, concept approval was obtained from the DCT Board of Scientific Counselors to invite proposals on the study of chronobiology and the influences of biorhythms on the efficacy and toxicity of cancer treatments.

Program Announcements

One of the most useful tools to stimulate research in specific areas is the solicitation of grant applications on particular topics. In FY88, announcements on improvements in the use of liposomes as delivery systems in cancer therapy and on specific cancer cell targeting using molecular genetic technology were developed. These joined the dozen or more program announcements that have been made in preceding years and that form the basis of the grant portfolio.

Contract Program

The contract program has been critically evaluated and nearly completely revamped in FY88. The BRB initiates requests for proposals (RFPs) and provides programmatic direction, evaluation, and monitoring for contracts supporting the mission of the BRMP. During FY88, the BRB awarded approximately $5.27 million for 18 extramural contracts, including $3.2 million in clinical task order contracts and $2.37 million in 7 non-clinical contract awards; 8 were new awards and 2 received incremental funding. Contracts provide for collection, storage, and quality assurance of BRMs; clinical development (including formulation) and distribution of BRMs; characterization and biochemical analysis of BRMs; chemical coupling of cytotoxic agents to monoclonal antibodies; generation of clinical grades of monoclonal antibodies; and procurement of BRMs for preclinical and clinical testing.

The BRB is also actively involved in the SBIR program. There are currently 5 active SBIR contract awards: 4 Phase II studies in the area of monoclonal antibody conjugates for therapy and diagnosis and 1 Phase I award for a databank on monoclonal antibodies.

Phase I/II Clinical Trials

In FY88, full-time Phase I/II contractors have been identified specializing in the performance of clinical trials of cytokines and lymphokines, and of monoclonal antibodies. The earlier master agreement mechanism will be scaled down to perform highly specialized clinical studies that ask distinct questions from those being addressed by the Phase I contractors. Current contracts are supporting clinical studies of monoclonal antibodies alone or with gamma interferon or interleukin-2 (IL-2); dose and schedule-related variations in the use of IL-2; I-131-conjugated T101 monoclonal antibody in T-cell lymphoma; gamma interferon alone and in combination with beta or alpha interferon; LAK cells plus IL2 with or without cyclophosphamide; monoclonal antibodies as immunomodulators, combined with standard therapies or armed with toxins; use of GM-CSF as a macrophage activator; and novel approaches to the generation of LAK cells.

The BRB clinical trials effort is closely linked with that of CTEP. Agents receiving initial evaluation in BRMP-sponsored Phase I studies are considered for Phase II and Phase II trials administered by CTEP through their extensive cooperative agreements with cooperative study groups. BRMP staff serve on the CTEP protocol review committee and CTEP staff serve on the BRMP operating committee. In addition, BCWG meetings are held weekly to coordinate the development of biological therapies.

Pharmaceutical Company Relationships

The BRMP has established relationships with the vast majority of biotechnology and pharmaceutical companies that produce BRMs. The BRB is currently expanding its relationships with the biotechnology industry by offering the opportunity to collaborate on joint programs for those BRMs with the greatest potential for clinical utility.

New Initiatives

The BRB is beginning the process of identifying Master Agreement holders to provide specialized products and services. For example, the synthesis of clinical amounts of clinical grade materials for clinical trials (peptides, oligonucleotides, recombinant molecules with novel functional moieties, etc.) and the performance of specialized immunological monitoring will enhance our capacity to conduct innovative clinical trials and obtain more valuable information from ongoing clinical studies.

Preclinical Evaluation Laboratory

The Preclinical Evaluation Laboratory performs experiments in two major areas: (1) on the use of biological agents to protect and restore marrow function with the aim of increasing the dose-intensity of chemotherapeutic agents with dose-limiting myelosuppressive effects; (2) on the development of complex therapeutic interventions employing combinations of biological therapies or biological therapies with chemotherapeutic agents rationally combined and monitored with surrogates of host and/or tumor response that will facilitate the rapid translation of preclinical treatment advances into the clinic.

The laboratory has demonstrated that the combination of IL 1 pretreatment and GM-CSF after treatment with cyclophosphamide or radiation converts and LD100 dose of the agents into and LDO. Up to 25% more radiation may be given when combined with marrow support. Experiments are ongoing to determine the optimal interval between the marrow stimulatory regimen and subsequent cycles of therapy. This question is critical for human studies that depend on repeated cycles of therapy since certain schedules result in augmentation of marrow damage by subsequent therapy rather than protection. For GM-CSF treatment, it appears that subsequent cycles may be safely given 7-10 days after cessation of GM-CSF administration.

The laboratory has been exploring the role of the hemoregulatory peptide HP 5b in marrow protection and restoration after chemotherapy and radiation. It appears that monomeric and dimeric forms of the peptide have distinct effects on the marrow and that appropriate timing of administration may be as protective as the best regimens employing hematopoietic growth factors such as IL 1, IL 3, and GM-CSF.

Experimental therapy of murine tumors has revealed remarkable synergy between IL 2 and the natural product, flavone-8-acetic acid, a flavonoid that appears to have potent capacity to stimulate the production of interferon alpha and perhaps a number of other cytokines. Experiments are underway to dissect the mechanism of the dramatic synergy observed between IL 2 and flavone acetic acid.

Biological Response Modifiers Program Operating Committee (BRMPOC)

The operating committee is the steering committee for the extramural program. It facilitates the development of biological agents by reviewing data generated by extramural investigators and commercial firms, organizing agenda items for the Division of Cancer Treatment Decision Network Committee (which sets the drug development priorities for the division), and provides review and general oversight of contract and grant program.

BRMP-Sponsored Meetings

This year the BRMP has sponsored or co-sponsored three highly successful scientific meetings: the Second Conference on Immunity to Cancer held in Williamsburg, Virginia, November 1987; a meeting on Specific Cancer Cell Targeting Using Molecular Genetic Technology held in Bethesda, Maryland in May 1988; and a meeting on the Development of Cytokines for the Treatment of AIDS held in Baltimore, Maryland in August 1988.

INTRAMURAL PROGRAM ACCOMPLISHMENTS

The intramural basic and clinical research efforts are highly integrated. There are numerous collaborations between labs and branches. There is an annual BRMP scientific retreat at which the progress of individual projects is openly reviewed and discussed. There are regular meetings of the scientists and clinicians in what is termed Therapeutic Applications Group meetings organized around topics ready to be tested in patients. These meetings provide a format for the basic scientists and clinicians to discuss findings and the design of clinical studies. There is abundant scientific exchange in weekly staff meetings and a monthly lab and branch chiefs meeting. There is an active seminar program at which intramural scientists and well-known extramural scientists from all over the world come to discuss their most recent findings. In addition, BRMP scientists are frequent organizers of national and international scientific meetings on basic and clinical science.

The intramural laboratories and branch have been introduced above. In addition to these units, the Associate Director maintains a separate laboratory effort.

Office of the Associate Director

There are two tenured senior investigators working independently within the laboratories of the Associate Director, Drs. Jonathan Ashwell and Ada Kruisbeek. Dr. Jonathan Ashwell has been studying signal transduction through the T-cell antigen receptor. His previous work demonstrated that signals, such as antigen plus self-MHC gene products, antibodies to the T-cell antigen receptor (Ti), antibodies to the nonpolymorphic protein complex associated with Ti (CD3), and antibodies to the Thy 1 antigen, which are all activation signals for normal T cells, can result in the death of malignant T cells both in vitro and in vivo. A clinical trial testing this approach to the treatment of T-cell lymphomas has begun. Dr. Ashwell has gone on to characterize the mechanisms by which tumor cells escape from the effects of activation signals and the resulting findings have dramatically altered our concepts of how the T-cell antigen receptor works. The CD3 complex consists of at least five polypeptide chains. The gamma, delta, and epsilon chains are primarily extracellular, and the zeta chain is present as a homodimer located primarily within the cell. Dr. Ashwell has shown that about 80% of the CD3 complexes in a given T cell contain zeta-zeta homodimers and 20% contain a newly described zeta-eta heterodimer. By analyzing signal transduction in T cells that lack one or the other of these complexes, Dr. Ashwell has shown that the zeta-zeta homodimer transduces signals through a tyrosine kinase pathway and the zeta-eta heterodimer transduces signal through phospholipase C. Thus, these data demonstrate a novel mechanism by which a single ligand binding molecule can initiate distinct and independent intracellular pathways through an association with different signal transducing elements.

Dr. Ada Kruisbeek has been examining the developmental biology of T cells and the mechanism by which the thymus exerts its influence over the T-cell repertoire for antigen. She has made enormous progress in the last year in formulating a picture of the enigmatic second class of T-cell receptor, the gamma-delta receptor. Through a careful analysis of families of T-cell hybridomas expressing the receptor and derived from different populations of cells (nude mice, thymocytes, etc.), she has found three different types of gamma chain and four types of delta chain are used to form the receptor, a diversity that had not previously been appreciated. In addition, there is nonstochastic association between certain variable region genes suggesting that there may be rules for the association of certain gene segments and there may be a restricted recognition of certain types of antigens by these enigmatic cells. Her studies of thymus function have been conducted both in vivo and in vitro. She has shown that class I-restricted T cells must interact with class I MHC molecules during their development to mature normally. In the absence of such interactions, a developmental arrest ensues and no class I-restricted cytotoxic T cells mature. She is now exploring ways of inducing the normal maturation of such arrested cells in vitro and is defining their developmental requirements. In a striking series of experiments, she has also demonstrated an essential role for the IL 2/IL 2 receptor pathway in the development of mature T cells, since the saturation and blockade of IL 2 receptor function in the developing fetus completely blocked the development of normal class I-restricted cytotoxic and class II-restricted helper T cells in vivo. She is now studying the requirements for the maturation of these cells in vitro in thymus organ culture.

In collaboration with Dr. James Kenny of Program Resources, Inc., the laboratory is also examining the effect of expression of a single intact immunoglobulin molecule specific for a defined antigen (phosphorylcholine) introduced into the germline of a mouse. These immunoglobulin transgenic mice have been extremely valuable tools to study B cell development, the rules governing the sequence of immunoglobulin gene rearrangement, normal B cell triggering by antigen, and the interactions between B cells and T-helper cells. For example, it has been learned that the membrane form of the transgene is capable of inhibiting the rearrangement of the endogenous immunoglobulin genes in over 95% of B cells, however, the secretory form of the gene does not make an impact on the developing B cell repertoire. About 5% of the B cells appear to express both endogenous immunoglobulin genes and the transgene. However, careful analysis of these cells reveals that the endogenous component is a truncated heavy chain, which can be produced without gene rearrangement. Studies are ongoing to analyze the interaction of the cells with antigen, T cells, and a variety of factors, cytokines, and lymphokines involved in B-cell activation in an effort to understand signal transduction mechanisms and control of proliferation and differentiation.

Laboratory of Molecular Immunoregulation

The Laboratory of Molecular Immunoregulation (LMI) investigates at a molecular level the intercellular and intracellular processes that regulate host defense mechanisms. The Office of the Chief administers research carried out by three investigators, Drs. Joost Oppenheim, Kouji Matsushima, and Scott Durum.

Dr. Oppenheim is a senior scientist of international stature who has had a distinguished career in the in vitro analysis of lymphokine action. This year he won two international research prizes and a Public Health Service medal for his excellence in research. More recently, he has turned his prodigious talents to the immunophysiology of lymphokines and cytokines in vivo. He and his colleagues made the remarkable discovery that pretreatment of mice with low dose of IL 1 about 20 hours before exposure to lethal doses of total body irradiation (LD100, 1000R) completely protects the irradiated animals. All animal survive an LD100 dose of radiation. More careful study has indicated that these observations also extend to lethal doses of alkylating agents like cyclophosphamide. Dr. Oppenheim is now applying these observations to clinically related problems of cancer treatment and allogeneic bone marrow transplantation.

Dr. Kouji Matsushima has been extraordinarily productive this year. He has performed experiments demonstrating profound synergistic effects between IL 1 and interferon-beta or between IL 1 and tumor necrosis factor in the induction of terminal differentiation of some primitive hematopoietic tumor cell lines. The effects of both combinations of synergistic cytokines can be blocked by antibody to interferon-beta. He and his colleagues have also begun to dissect the complicated regulation of expression of IL 1 receptors which are expressed on nearly every cell type. He has also begun investigating the intracellular consequences of IL 1 interacting with its receptor. He has identified a number of important events that occur within the cell including induction of ornithine decarboxylase, induction of manganese superoxide dismutase, and the induction of an as yet unidentified phosphoprotein of 65K molecular weight. He has found that the intracellular events that occur in response to IL 1 are in several ways related to the cellular response to tumor necrosis factor and IL 6 (formerly interferon-beta2). Evidence is accumulating that these three cytokines have considerable overlap in their activity and it promises to be very important to dissect how they differ and how they are regulated. Finally, Dr. Matsushima (in collaboration with NCI colleagues, especially Dr. Edward Leonard's laboratory) has identified, sequenced and cloned a novel cytokine called monocyte-derived neutrophil chemotactic factor. The factor is unique, is induced by monocyte activating agents like IL 1 and tumor necrosis factor, and the recombinant molecule expressed by E. coli is active in vivo in mice to stimulate neutrophil chemotaxis. This molecule may be of use in the management of cancer and infectious diseases.

Dr. Scott Durum has performed a series of studies that challenge the dogma of macrophage-T cell interaction. First, it was felt that among the signals macrophages provide to T cells was IL 1, to which the T cells may respond by proliferating. Dr. Durum has demonstrated that many T cells not only respond to IL 1 but produce their own. Thus, he added IL 1 to IL 2 on the list of factors made by T cells that act in an autocrine fashion to stimulate T-cell proliferation. Second, it was thought that the interaction between macrophages and T cells was essentially one-way with the macrophage acting on the T cell and the T cell responding. Dr. Durum has demonstrated that the T cell also exerts important effects on the macrophage. For example, the T cell produces what appears to be a novel lymphokine that stimulates the macrophage to produce IL 1. In addition, the T cell produces a factor that induces a surface structure on the macrophage that enhances or stabilizes the interaction between the macrophage and the T cell. If one prevents the macrophage from expressing this T-cell induced surface structure, no T-cell activation can occur and soluble products of the macrophage cannot replace the cell interaction. Thus, Dr. Durum's work has fundamentally altered the popular concept of macrophage-T cell interactions and is helping to clarify and expand our understanding of these critical first steps in the cellular immune response.

Dr. Howard Young and Dr. Luigi Varesio are the senior investigators in the Immunobiology Section of the LMI, which conducts studies on the intracellular events responsible for the differentiation of lymphocytic and monocytic cells and their activation to antitumor effectors. In addition to his primary investigations, Dr. Young collaborates widely in the BRMP to provide molecular biology expertise to colleagues whose problems require such tools. Dr. Young's primary interest has been the regulation of expression of the human interferon gamma gene. Although it is called an interferon, interferon gamma differs greatly from the alpha and beta species. Among the differences that Dr. Young is investigating is the highly restricted tissue specificity of interferon gamma production. The only two cell types known to produce interferon gamma are T cells and large granular lymphocytes (LGLs, usually with NK activity). Dr. Young is elucidating the mechanism of the tissue specificity of interferon gamma production through a series of ingenious experiments in which he introduces the human genes into a variety of murine recipients and evaluates the control of human interferon gamma expression. He has defined the parts of the gene responsible for promoting expression and has identified an entirely novel mechanism of post-transcriptional gene regulation, failure to transport message from the nucleus to the cytoplasm for translation. In addition to these studies, Dr. Young has been examining the role of methylation in the regulation of expression and rearrangement of T-cell receptor genes. He has found the genes to be hypomethylated in T cells, slightly more methylated at particular GC-rich sequences in LGLs (which express an aberrant 1 kb message that does not appear to be translated), and to have the highest degree of methylation in the T-cell receptor genes from B cells and macrophages that transcribe no gene product. Thus, methylation may be a mechanism of regulation of T-cell receptor gene expression in particular cell lineages.

Dr. Luigi Varesio has been focussing his efforts on the biochemical events associated with the activation of macrophages to become cytotoxic antitumor effector cells. He has established immortalized bone marrow macrophage cell lines by infecting normal bone marrow with a recombinant retrovirus containing both myc and raf oncogenes. Cells of different stages of differentiation emerge from the infection of marrow from distinct strains of mice, however, the cell lines respond to macrophage activating agents by the cessation of spontaneous proliferation and the differentiation to cytotoxic macrophages. In addition to differentiating into tumor killers, these cells also decrease their expression of transforming oncogene mRNA in response to interferon gamma, and the mechanism appears to relate to a decreased stability of the viral message. The basis for this enhanced turnover of oncogene message in response to interferon gamma is under investigation and could have therapeutic applications. In addition to the studies on the effects of interferon on oncogene expression, Dr. Varesio has shown that macrophages respond to interferon gamma through a distinct pathway than their response to interferon alpha or beta, though all forms of interferon lead ultimately to cytotoxic differentiation and before that to an alteration in the processing of ribosomal RNA. Interferon gamma activation is independent of protein synthesis and protein kinase C, but interferon alpha and beta both require protein synthesis and protein kinase C to lead to cytotoxicity. Dr. Varesio believes that the altered ribosomal RNA processing is critical to the differentiation since ribosomal RNA precursors are capable of forming double-stranded segments that activate double-stranded RNA-dependent enzymes, which appear to be critical to the effects mediated by interferon. Ribosomal RNA appears to play a primary role in interferon-mediated intracellular events.

Drs. William Farrar and Frank Ruscetti are the senior investigators in the Lymphokines section of the LMI, which investigates the mechanism of action of lymphokines and cytokines at the molecular level. Dr. Farrar is one of the BRMP's most productive and innovative scientists. He has performed a large number of studies dissecting the intracellular events that occur as a consequence of a particular growth factor interacting with its specific receptor on a cell that is dependent on that factor for its growth. For example, he has demonstrated that the activation pathways are similar in IL 2-dependent cells responding to IL 2, IL 3-dependent cells responding to IL 3, and GM-CSF-dependent cells responding to GM-CSF. The common activation pathway bears a close resemblance to the events occurring in lower cell forms (e.g. bacteria and yeast) in response to heat shock or other oxidative stress and include activation of two distinct kinase systems, induction of a 68kd phosphorylated substrate, inhibition of adenylate cyclase activity, activation of GTP binding proteins, induction of heat shock proteins, synthesis of a phosphorylated diadenylate (Ap4A) which facilitates DNA polymerase alpha activity, and phosphorylation of a 40S ribosomal protein called S6 which regulates the rate of protein elongation during protein synthesis. He is now pursuing the regulation of DNA binding proteins that may be involved in orchestrating this cascade. The implications of his work are that there are a limited number of ways for a cell to respond to stimulation. Specificity of response appears to be an adaptation to trigger a stereotyped response to the specific interaction of a factor with its receptor. After receptor binding, the similarities in the cellular events are striking. Dr. Farrar has also been interested in elucidating the intriguing interrelationships between the immune system and the nervous system. He has demonstrated that a number of receptors thought to be restricted to the immune system are also found in particular anatomic sites of the brain, including the first demonstration of a non-T-cell source of IL 3, the cerebellum. Conversely, he has shown that many neuropeptide receptors, such as the beta-endorphin receptor, are expressed on lymphoid cells. He has shown that the neuropeptide receptors on lymphocytes are functional and the immunologic receptors on neurons are also functional. For example, signal transduction through the CD4 molecule on neuroblastomas cell lines results in differentiation into cells with neuronal morphology. Thus, there appears to be a reciprocal network of shared ligands and receptors between the nervous system and the immune system and undoubtedly there are important overlaps in function and regulation that will be found by further study.

Dr. Frank Ruscetti is studying the molecular regulation of hematopoiesis. He has studied the induction of specific oncogenes as a consequence of the interaction of hematopoietic growth factors with their receptors in normal and transformed cells. He has demonstrated that hematopoiesis is controlled not only by stimulatory factors and positive regulation, but in addition, transforming growth factor-beta (TGF-beta) acts as a negative regulator inhibiting the growth of primitive multipotential bone marrow cells. This effect is quite specific since unipotent bone marrow colonies are not affected by TGF-beta, due the fact, as Ruscetti has shown, they do not express TGF-beta receptors. Dr. Ruscetti has also demonstrated the potential therapeutic value of TGF-beta in certain human hematopoietic and lymphoid tumors. All primitive human leukemic cell lines are potently growth inhibited by TGF-beta. Although lymphoid tumor cells are not, they can be induced with a variety of agents to express TGF-beta receptors, and when they do, they are growth inhibited by it. In addition, it appears that HL-60 cells induced to differentiate are growth inhibited because they are also induced to express and respond to TGF-beta. Thus, TGF-beta is implicated in the genesis of certain neoplastic conditions (i.e. escape from normal growth regulation via down-regulation of the TGF-beta receptor) and may be therapeutic in a number of clinical settings. The capacity of TGF-beta to protect bone marrow progenitors from damage by chemotherapeutic agents is also under investigation.

Another area of investigation for Dr. Ruscetti is the regulation of replication and the mechanism of transformation by human retroviruses. Dr. Ruscetti was involved with the discovery of both IL 2 and the first human retrovirus, HTLV-I. It has been a puzzle to understand how HTLV-I can be responsible for the neoplastic transformation of mature T cells to produce the disease adult T-cell leukemia when the fresh tumor cells rarely if ever are producing virus or viral mRNA. Dr. Ruscetti has demonstrated that a number of HTLV-I-transformed cell lines express unusually high amounts of the rel oncogene. He has collected evidence to suggest that the primary infection of a cell with HTLV-I can lead to the expression of certain host genes including rel, which are then responsible for the transformation process. Dr. Ruscetti has evidence that a variety of host cellular genes can interact with the viral genome and some of the effects can be to inhibit viral replication. He is now pursuing the nature of the gene product that interferes with viral replication, a molecule that may have therapeutic application in certain lymphoid tumors and in AIDS.

Laboratory of Experimental Immunology

The Laboratory of Experimental Immunology (LEI) conducts studies on biological response modification and the application of these studies to the treatment of cancer. Dr. John Ortaldo, Chief of the LEI, conducts his own personal laboratory work under the Cellular and Molecular Immunology Section (CMIS) headed by Dr. Craig Reynolds.

Drs. Ortaldo and Reynolds continue to work closely together on the mechanism of cell recognition and lysis by LGLs both in their native state (i.e. as NK effectors) and activated by IL 2 (i.e. as LAK effectors). The laboratory has developed an anti-idiotypic antibody that reacts primarily with LGLs and has several important physiologic functions: both intact antibody and F(ab')2 fragments block binding of LGLs to their targets (K562), block lysis of targets, and induce significant activation of the cells upon preincubation with antibody. It immunoprecipitates an 80kd molecule which is the candidate LGL target receptor and this receptor is currently being characterized. LGL are also potent producers of a panoply of cytokines. In fact, work from CMIS has led to the proposal that much of the antitumor activity (and perhaps toxicities) of LAK activity in vivo is related to the production of lymphokines by these cells. Unlike T cells, it has been found that LGLs will secrete their cytokines in response to a single stimulus like IL 2. T cells usually require two activation signals. A number of potentially cytotoxic molecules are secreted by LGLs including TNF, cytolysin (a 60 kd granule protein), interferon alpha, interferon gamma, lymphotoxin, and a newly described factor called NKCF (natural killer cytotoxic factor). Antibodies to NKCF react with a 12 kd protein and completely block NK activity in vitro suggesting an important role in NK function. NKCF is also effective in lysing fungi. Its mechanism of killing and target recognition are under investigation. Several recent in vivo studies have laid to rest the old question of whether LGLs with NK activity had an important role in host defense. Adoptive transfer of LGLs into rats with depressed NK activity restored in vitro cytotoxicity of peripheral blood lymphocytes, increased in vivo clearance of tumor cells from the lungs and led to resistance to experimental lung metastases. In addition, it appears that NK cells play an important role in the resistance of animals and man to engraftment from both syngeneic and allogeneic sources. LGLs are potent inhibitors of hematopoiesis in vitro (possibly because they produce TGF-beta), and recent experiments from the CMIS demonstrate that in vivo depletion of LGLs have a dramatic effect to enhance engraftment of syngeneic and allogeneic marrow. These studies have led to the formulation of a clinical trial in which LGL-depletion will form a component of the host preparation regimen before transplantation. If successful, this could greatly enhance the control of GvH disease since fewer bone marrow cells (and thus, fewer contaminating T cells that mediate GvH) will be required to reconstitute the transplant recipient. There are also experimental protocols underway to use host LGL depletion to facilitate tolerance induction to foreign MHC antigens and prepare recipients for organ transplants.

Another effector function of LGLs is antibody-dependent cellular cytotoxicity (ADCC). The CMIS investigators have found that most ADCC activity in mixed populations of human effector cells resides in the LGL subset and that among murine immunoglobulin isotypes, IgG3 are the most efficient activators of ADCC. ADCC activity was dramatically enhanced by interferon alpha or gamma and by IL 2. Preclinical animal models are under evaluation and human clinical trials of monoclonal antibodies plus interferon or plus IL 2 have begun.

The Leukocyte Differentiation Section (LDS) under Dr. John Ortaldo as acting head is comprised of a single senior investigator, Dr. Bonnie Mathieson and serves as the expert source of new monoclonal antibodies for the program and it studies the differentiation and activation of human and murine lymphocytes. Dr. Mathieson has used intrathymic transfer of enriched cell populations to demonstrate that the thymocyte stem cell is a CD5 dull, CD4 negative, CD8 negative population that comprises less than 5% of the total thymocyte population. However, on intrathymic transfer, this subset can completely reconstitute the various normal thymocyte populations. A similar system has been used to study LGL ontogeny. Intravenous injection of LGLs or bone marrow (as a source of precursors) results in donor-derived LGLs, but if the cells are injected intrathymically, only the bone marrow cells are capable of reconstituting the periphery as measured by liver-associated LGLs. Furthermore, the use of the same CD5 dull double-negative subset that gave rise to mature thymocytes also gives rise to peripheral LGLs. This is the first evidence that LGLs and T cells have a common bone marrow-derived progenitor.

The LDS has also been responsible for the generation of a number of important monoclonal antibodies that are serving as probes for the function of important molecules. For example, the laboratory generated an antibody specific for murine LGLs that may be more useful at defining the LGL subset and its function than the heterologous polyclonal and nonspecific anti-asialo GM1. In addition, in collaboration with Dr. Steven Giardina of Program Resources, Inc., monoclonal antibodies to the fos and raf oncogene products have been generated. These antibodies are being used in studies to assess the role of these oncogenes in cell proliferation and differentiation.

The Experimental Therapeutics Section (ETS) under Dr. Robert Wiltrout evaluates the antitumor efficacy of BRMs and studies the mechanisms by which these agents function in vivo. Dr. Jack Pearson is a senior investigator within the section. Dr. Wiltrout has devised some extremely effective chemoimmunotherapy and adoptive chemoimmunotherapy protocols for the treatment of advanced stages of solid tumors in mice. His previous work on the bicompartmental administration of doxorubicin, LAK cells and IL 2 have formed the basis for an ongoing clinical protocol in the Clinical Research Branch. More recently, he has been exploring the mechanism by which the investigational drug, flavone-8-acetic (FAA) synergizes with IL 2 in the treatment of an advanced stage renal cancer in BALB/c mice. He has found that FAA is a potent inducer of organ-associated NK activity. Since it appears that the majority of LAK activity comes from NK precursors, the fact that the precursors migrate and expand in the spleen, liver, lungs, and peritoneum set the stage for in vivo activation by systemic IL 2 administration. FAA also is a potent inducer of interferon alpha. However, administration of interferon alpha with IL 2, though modestly effective, is not nearly as synergistic as FAA plus IL 2. A Phase I/II clinical trial of FAA plus IL 2 based on Dr. Wiltrout's work is also underway. The ETS has also been examining the mechanism of augmentation of organ-associated NK cells after administration of certain BRMs such as FAA. Ablation of bone marrow cells interferes with the accumulation of NK cells in the organs, thus, it appears that most of the effects of BRMs is not related to a redistribution of pre-existing NK cells but a directed homing of newly generated NK cells coming from the marrow. Studies to understand the mechanism of this directed trafficking are in progress. Direct injection of certain BRMs into the peritoneal cavity (e.g. TNF or IL 1 or interferon gamma) results in accumulation of NK cells in the peritoneal cavity, but the cells are not activated without the addition of exogenous IL 2. Preliminary experiments on the mechanism of action of this NK chemotactic effect appear to point to an effect on vascular endothelium. Leukotrienes and prostaglandins appear to be uninvolved.

Dr. Wiltrout and his colleagues are also developing models of tumors in vivo that will allow an assessment of interventions aimed at interfering with oncogene expression. For example, Dr. Varesio's macrophage cell lines infected with raf and myc are tumorigenic. Pilot studies are underway to evaluate mechanisms of delivery of antibodies or antisense oligonucleotides for therapeutic purposes.

Dr. Jack Pearson has been evaluating combined chemotherapy plus immunotherapy in human tumor xenograft models. He has established conditions for the in vivo growth of both ovarian and colon tumor lines that mimic the human disease. He uses combination chemotherapy to induce an initial response in animals with established tumor and is evaluating immunotoxins and biological agents with direct antitumor effects (rather than indirect effects on the host) to define complex therapies that may be effective in man. He has found that the use of WR-2721 to ameliorate cyclophosphamide toxicity has allowed higher doses to be given and has allowed the subsequent immunologic approaches (ricin and Pseudomonas exotoxin immunoconjugates) to be more effective in their 2-3 log cytoreduction. Significant prolongation of survival has been obtained.

The Laboratory of Biochemical Physiology

The Laboratory of Biochemical Physiology (LBP) under the direction of Dr. Hsiang-fu Kung provides general molecular biology and biochemistry expertise to the other investigators in the BRMP and is charged with performing studies on the genetic and biochemical events related to the development and expression of the malignant phenotype. In the two short years of its existence, the LBP has quickly become an indispensible part of the program and a scientific entity that contributes to the literature out of proportion to its small size. The laboratory has performed elegant studies on the structure/function relationships between the ras oncogene family of proteins and their biological effects. For example, Dr. Kung has shown that the normal ras protein is an integral part of the normal development of Xenopus since interfering with ras function arrests development. He has shown that normal ras protein stimulates phosphoinositide (PI) turnover via a distinct signal transduction pathway than platelet-derived growth factor and that the oncogenic mutant ras protein exerts no effects on PI turnover. By performing microinjection studies in the Xenopus oocyte system and studying the effects of various signals on normal maturation, Dr. Kung has shown that the oncogenic ras protein induces oocyte maturation and protein kinase C potentiates insulin-induced maturation but does not itself induce maturation. Both signals result in ribosomal S6 protein phosphorylation suggesting that this is an important step that several distinct pathways may converge upon.

The laboratory's interest in ras extends to other G proteins as well. For example, the LBP was the first to molecularly clone the Go alpha member of this family, which is increased in normal brain, malignant neuronal and neuroendocrine tumors, and endocrine cells in the adrenal, pituitary, thyroid and pancreas, and has been suggested to be involved in coupling opiate receptors to calcium mobilization in cells that express it. The sequence is now being used to survey a number of tumor types in which it may be a molecular marker.

The LBP has performed some very provocative studies using microinjection technology in collaboration with Drs. Scott Durum of the LMI and Mark Smith of

Program Resources, Inc. Although most cytokines have cell surface receptors that mediate their action, there are also trophic cell stimuli whose receptors are cytosolic, e.g. vitamin D receptors, steroid receptors. The presence of a cell surface receptor does not preclude an intracellular target. Therefore, the LBP has been evaluating the trophic effects of microinjected cytokines. Intracellular injection of IL 2 and IL 1 had no apparent effects. However, microinjection of human interferon gamma into murine macrophages resulted in the induction of MHC class II molecules not only on the injected cells but also on adjacent uninjected cells. Since the murine cell surface receptor for interferon gamma does not bind human interferon gamma, these results cannot be explained by a leakage of human interferon gamma out of the cell and its interaction with the cell surface receptor. The data suggest that human interferon gamma interacts with a cytosolic receptor that leads to the induction of MHC gene expression. The effects on uninjected cells suggest that perhaps the injection also induce the production of another class II MHC-inducing cytokine. Blocking studies demonstrated that antibody to GM-CSF prevented the induction of class II MHC molecules on uninjected cells. Therefore, it appears that GM-CSF production is also a consequence of the interaction of human interferon gamma with an intracellular target structure.

In collaboration with Dr. Connie Faltynek of Program Resources, Inc., the LBP has been studying the mechanism of action of the interferons at the level of interaction with cell surface receptors and signal transduction. The interferon alpha receptor has been found to be a 105 kd molecule that is down-regulated upon exposure to interferon alpha. In addition, cross-linking studies reveal a 90 kd molecule that is not apparently under interferon autoregulation. Although it is clear that induction of certain enzymes such as 2-5 oligo-adenylate synthetase and other proteins are the result of a cell being stimulated with interferon alpha, the LBP has shown that these end results depend on signal transduction through a functional protein kinase C. However, protein kinase C agonists such as phorbol esters do not mimic the effects of interferon. Thus, protein kinase C is necessary but not sufficient to initiate interferon action and other intracellular signal transduction pathways are likely to be involved as well.

AIDS-Related Research in BRMP Intramural Laboratories

Each of the intramural laboratories has ongoing research projects related to AIDS. The BRMP AIDS research effort has three major components: 1) to understand the regulation of virus production and viral pathophysiology; 2) to attack the virus by a molecular or immunological strategy; and 3) to reconstitute the immune system after viral replication is controlled.

1. To understand the regulation of virus production and viral pathophysiology

The BRMP is studying the genetic mechanisms that regulate viral replication in T cells and monocytes and has discovered that viral latency occurs by distinct mechanisms in the different cell types. In T cells, viral latency appears to be related to host cell DNA binding proteins that recognize the viral LTR. These DNA binding proteins are being purified and characterized. In monocytes, the virus replicates and forms intact virions intracellularly but the latency is related to extrusion of intact virions from the cell. Various signals that activate macrophages also increase the production of virions. Efforts are underway to characterize the defect in the process of viral extrusion that seems to be at the heart of the latency of lentiviruses in general and the AIDS virus, HIV, in particular.

Efforts are also underway to better understand the pathophysiology of the virus. For example, BRMP investigators have discovered that viral proteins are capable of inducing trophic effects on a number of different kinds of cells (monocytes and neural cells). At least some of the clinical manifestations of the disease may be mediated by direct stimulation by viral proteins. In addition, since some of the organ effects in AIDS resemble autodestructive lesions of the immune system rather than immunodeficiency, mechanisms of self-tolerance and suppression are being investigated for their possible role in the disease manifestations. Finally, insights into pathophysiology are being sought by examining both lymphoid and hematopoietic cell development in a murine AIDS model.

2. To attack the virus by a molecular or immunological strategy

The BRMP is exploring a number of novel approaches to attack the virus that might complement the antiviral agents that have been demonstrated with AZT and other dideoxynucleosides. For example, certain lymphokines appear capable of significantly reducing the number of CD4 molecules on the surface of cells that are targeted by the virus. Some biological signals can alter viral replication. HIV envelope analogs may interfere with HIV binding to its target. Certain peptides have been identified that affect viral infectivity without binding to CD4 or competing with gp120 binding. Sorting out the cellular mechanisms of these inhibitors may result in a new treatment strategy.

It is clear that some patients with HIV infection have some serum antibody (perhaps anti-gp120) that successfully blocks virus infection and BRMP scientists have demonstrated that NK cells and LAK cells are very potent at lysing HIV-infected cells. Thus, strategies that boost host immunity to the virus may be clinically useful.

Finally, the evidence from monocytes that are latently infected with HIV is that viral production may depend crucially on glycosylation pathways in the host cell and particular intracellular compartments may allow specific attack on HIV replication and production.

3. To reconstitute the immune system after viral replication is controlled

The BRMP is examining thymus and bone marrow transplantation in addition to the effects of a number of cytokines (including a novel lymphokine made by thymocytes) in an effort to regenerate a functional immune system after HIV replication is controlled.

The Clinical Research Branch

The Clinical Research Branch (CRB) under Dr. Ronald Steis includes Drs. John Smith and Jeffrey Clark as senior investigators. The CRB is responsible for the clinical testing of biological therapies, their integration with other modalities of cancer treatment, and the correlation of in vitro parameters of immune and biological function with in vivo antitumor effects. The CRB consists of a 10-bed outpatient unit in the Medical Pavilion, 335 Park Avenue, Frederick, a 13-bed inpatient unit in the Frederick Memorial Hospital that includes a 4-bed intensive monitoring unit, and a 4-bed pheresis unit.

The clinical interests of the CRB are diverse. The clinical effort depends heavily on extremely close collaboration with Dr. Walter Urba and his colleagues in the Clinical Immunology Laboratory of Program Resources, Inc., perhaps the finest clinical monitoring laboratory of its kind in the world. Currently there are active protocols testing recombinant cytokines alone and in combination, recombinant cytokines with adoptive cellular therapies, recombinant cytokines with single agent chemotherapy, recombinant cytokines with classic BRMs, and recombinant cytokines in combination with cytokines, adoptive cellular therapy and combination chemotherapy. There are also active protocols evaluating immunotoxins and human monoclonal antibodies for imaging and treatment, and the use of low doses of anti-CD3 antibody as an antitumor agent in T-cell malignancies and an immune stimulant in patients with other types of tumors.

In the last year the CRB has published a number of important findings. They found that 15/51 patients with hairy cell leukemia treated with chronic low-dose interferon alpha developed neutralizing antibodies to the treating interferon, but in vitro these antibodies did not block the antiviral effects of a mixture of alpha interferon species. They were specific for the treatment interferon alpha 2a. Six of the 15 patients with antibodies developed interferon resistance. No patient without antibody was resistant to the therapeutic effects of interferon. This study is important because it suggests that patients who lose responsiveness to interferon may not have tumor cells resistant to interferon but may be manifesting resistance on a pharmacokinetic basis. Furthermore, it appears that switching treatment to another species of interferon may result in a reappearance of disease control. Since the interferon used to treat the patients who developed antibody is identical to the endogenously-generated interferon alpha 2a, these results also suggest that somehow chronic treatment with interferon led to a breakdown of self-tolerance. It remains to be seen whether this will be a general problem with recombinant molecules aimed at enhancing immune function.

Two other important studies in hairy cell leukemia were also published. The CRB researchers were the first to demonstrate that nonsplenectomized hairy cell leukemia patients who are refractory to interferon alpha treatment are often responsive to 2'-deoxycoformycin. They also reported the first convincing tumor marker for hairy cell leukemia, serum soluble IL 2 receptor levels. The IL 2 receptor levels in the serum go down in responding patients and go up in the face of progressive disease even before the recurrence of cytopenias. Early therapeutic intervention is indicated in a hairy cell leukemia patient with increasing levels of serum soluble IL 2 receptor.

The CRB also completed a clinical trial in hairy cell leukemia patients that was an effort to obtain a higher complete response and a more durable remission. Patients were treated with alternating interferon alpha and 2'-deoxycoformycin in alternating monthly treatment cycles. When complete remission is defined as negative bilateral bone marrow biopsies repeated with an interval of 1 month, it was found that all the patients had partial responses. Previous definitions of complete remission were clearly less rigorous and were associated with short remission durations. There is no evidence that the combination of interferon and deoxycoformycin is superior to either agent alone. On the other hand, it has been found that patients treated with deoxycoformycin develop profound defects in T-cell functions, and the immune dysfunction lasts a minimum of 6 months after discontinuing the therapy. A few cases of herpes zoster have been documented, but nationwide only a single serious opportunistic infection has been observed in a deoxycoformycin-treated patient.

The CRB was the first to administer adoptive therapy intraperitoneally. A clinical trial with intraperitoneal monocytes activated with interferon gamma has been completed and found to be safe. A subsequent clinical trial to assess antitumor efficacy is now underway. In addition, intraperitoneal LAK cells plus IL 2 have been administered to 23 patients with colon cancer or ovarian cancer restricted to the peritoneal cavity. The overall partial response rate was 34%. All responding patients were retreated, however, none converted to complete responders and all developed treatment-related toxicity that prohibited continuing therapy. The limiting toxicity was intraperitoneal fibrosis, often necessitating surgical intervention. Other clinical trials of systemic and intraperitoneal adoptive cellular therapies have now been begun.

The CRB also demonstrated the optimal immunomodulatory dose for interferon gamma and is now testing the antitumor effects of that dose administered over several months (unless tumor progression occurs). The BRMP study was the first to document the often predicted bell-shaped dose response curve. This made it clear that not all biological agents are likely to be optimally effective when pushed to dose-limiting toxicity. The challenge before us the the integration of biological therapies with other treatment modalities.

A first step to integrating therapies was a BRMP Phase I study of recombinant human GM-CSF. The molecule produced a dose-related increase in peripheral blood granulocyte and monocyte counts that continued to increase as long as the drug was given. No dose-limiting toxicity was found. Unfortunately, the supplier (Immunex) ran out of drug before an MTD could be determined. At the doses and schedule given, no macrophage activation was detected but the induction of adhesion molecules on the surface of the granulocytes was observed.

The next study will attempt to use the GM-CSF to support delivering an increased dose-intensity of chemotherapeutic agents in a setting where there is evidence that more drugs will result in a higher cure rate. MOPP combination chemotherapy will be dose escalated with the support of GM-CSF in an effort to improve the treatment outcome of patients with Stage IIIB or IVB disease.

The ongoing studies with IL 2 given in an effort to maintain high levels of Leu 19 positive cells in the peripheral blood and used in combination with the interferon inducers, flavone-8-acetic acid and poly I:C-LC should demonstrate whether IL 2 responses can be improved in frequency or duration by inducing endogenous interferon alpha production.

One of the most interesting ongoing clinical studies combines LAK cells with IL 2, chemotherapy with doxorubicin plus cyclophosphamide, and interferon alpha plus aspirin. Each component of the therapy has a 15-30% response rate. It is hoped that the components have been integrated in a fashion that will augment the single component response rates.

The BRMP has been the first clinical group to perform imaging studies using human monoclonal antibodies. Unfortunately, the images are not superior to those generated with murine antibodies, however, unlike murine antibodies, no human immune response was elicited. Future studies will be aimed at using the human antibodies for therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09290-03 OAD

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Antigen Presentation and T-Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. D. Ashwell	Senior Investigator	OAD, NCI
Others:	M. Mercep	Visiting Fellow	OAD, NCI
	S. Bridges	Cancer Expert	MB, COP, NCI
	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS (if any)
Laboratory of Cellular and Molecular Biology, OBRR/CDB (P. Noguchi), Laboratory of Immunology, NIAID (M. Jenkins and R. Schwartz).

LAB/BRANCH
Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
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CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The phenomenon of activation-associated growth inhibition of transformed T cells, both in vitro and in vivo, has been studied. In vitro. Activation of transformed T cells results in lymphokine production and inhibition of growth. Growth inhibition, as manifested by a decrease in [3H]thymidine incorporation and a G1/S cell cycle block, is manifested within 1 hr of stimulation. Removal of extracellular Ca2+ with EGTA completely blocks lymphokine secretion but has no effect upon early growth inhibition. However, the cell lysis that usually results 4 to 6 hr after stimulation was blocked by the removal of extracellular Ca2+. Cyclosporine A (CSA), which may exert its effects by antagonizing calcium-dependent intracellular pathways, had effects similar to EGTA. Moreover, in prolonged cultures in CSA (up to 48 hr) it was found that the early G1/S block was reversible. These data indicate that activation-associated growth inhibition is a two step process. The first, a G1/S cell cycle block, does not require extracellular Ca2+, is CSA-resistant, and is reversible. The second, cell lysis, requires extracellular Ca2+ and is CSA-sensitive. In vivo. Treatment of mice bearing antigen-specific T-cell tumors with the appropriate antigen results in tumor elimination. This is due to (1) the direct cytotoxic effect of antigen-stimulation on T-cell growth, and (2) the induction of host immunity. It was found that mice "cured" of one T-cell hybridoma subsequently rejected the same or related T-cell tumors. Cultured splenocytes from these mice were cytotoxic to the original tumor cell in vitro. Adoptive transfer of splenocytes indicated that Thy-1-positive cells (T cells) were responsible for the immunity. Furthermore, in vivo depletion of either the CD4+ or the CD8+ T-cell subset suggested that the latter, which contain mostly cytotoxic T cells, were critical in this response. These studies increase our understanding of activation-associated growth inhibition, and should enhance our ability to manipulate the phenomenon in vivo.

PROJECT DESCRIPTION

PERSONNEL

Jonathan D. Ashwell	Senior Investigator	OAD, NCI
Mladen Mercep	Visiting Fellow	OAD, NCI
Sandra Bridges	Cancer Expert	MB, COP, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

(1) To study the mechanisms by which activation results in cell cycle block and growth inhibition of transformed T cells;

(2) To develop reagents that will act as a surrogate for antigen in the activation of a wide variety of lymphocytic neoplasms;

(3) To explore in vivo models and develop methods of eliminating T-cell neoplasms of unknown antigen-specificity from the tumor-bearing animal; and

(4) To understand what signals provided by APC are required for the successful presentation of antigen to T cells, especially with regard to the deleterious effects that gamma radiation and oxidizing agents have on this function.

MAJOR FINDINGS

I. Activation-Associated Growth Inhibition of Transformed T Cells

We have previously demonstrated that within 24 hr of activation, transformed T cells produce lymphokines and stop dividing. Inhibition of growth is not mediated by the lymphokines, but is a direct consequence of activation. Furthermore, activation induced an apparently irreversible G_1/S cell cycle block and, within 6-8 hrs, cell lysis. We have further characterized these phenomena. By using 1 hr "pulses" of [^{3}H]thymidine, it was possible to assess the effects of various mitogenic stimuli on the growth of a T-cell hybridoma at very early time points. Using either anti-CD3, anti-Thy-1 or anti-clonotypic mAbs, or the lectin Con A, it was found that a substantial decrease (up to 40%) in [^{3}H]thymidine incorporation occurred within 1 hr of stimulation. This gradually increased with time, going to ~70% by 9 hr. Because of the well documented effects of extracellular Ca^{2+} depletion on lymphokine release, we had previously attempted to study the role of Ca^{2+} flux in signaling for growth inhibition. Unfortunately, depletion of extracellular Ca^{2+} was toxic to the cells over the course of the typical 24 hr assay. However, with the observation that growth inhibition could be reliably quantitated within a few hours of stimulation, experiments were undertaken in which EGTA was used to chelate extracellular Ca^{2+}. Surprisingly, whereas stimulation yielded no IL-2 under these conditions, the decrease in [^{3}H]thymidine incorporation was generally unimpaired. Furthermore, cell cycle analysis performed 5 hrs after stimulation found that a G_1/S cell cycle block was established equally well in the presence or absence of external Ca^{2+}. Thus, unlike lymphokine secretion, activation-associated growth inhibition does not require extracellular Ca^{2+} and, therefore, transmembrane Ca^{2+} flux. The effect of CSA, which may exert its effects by antagonizing Ca^{2+}-dependent pathways, was also assessed. As with EGTA, CSA blocked activation-induced secretion of IL-2.

In addition, after 6 hrs the decrease in [^{3}H]thymidine incorporation and the G_1/S cell cycle block were identical in the presence or absence of CSA. However, by 24 hr, the growth of cells stimulated in the presence of CSA was only inhibited ~50% as much as cells grown in the absence of CSA, and by 48 hrs the growth of the two populations was almost identical. This result was better understood when cell lysis, as measured by the release of ^{51}Cr was measured. In medium alone, 9 hrs after stimulation the hybridoma cells exhibited ~60% specific ^{51}Cr release. In contrast, identically stimulated cells grown in CSA did not release ^{51}Cr at all. To determine if it was CSA's effects on Ca^{2+}-dependent processes that was responsible for this, the effect of EGTA on cell lysis was analyzed. Unfortunately, the non-specific release of ^{51}Cr from cells grown without Ca^{2+} becomes prohibitively high by 6 hrs, the first time at which activation causes measurable ^{51}Cr release. However, trypan blue exclusion was found to be an earlier indication of cell lysis. Analyses performed 4 hrs after plating determined that stimulation with anti-CD3 mAbs causes ~20% specific trypan blue uptake. In the presence of EGTA, however, no specific trypan blue uptake could be measured. The addition of stoichiometric amounts of $CaCl_2$ resulted once again in ~20% specific trypan blue uptake. Based on these results, we now believe that activation-associated growth inhibition has two distinct phases. The first, a G_1/S cell cycle block, does not require extracellular Ca^{2+}, is CSA-resistant, and is probably reversible. The second, cell lysis requires extracellular Ca^{2+}, is CSA-sensitive, and, of course, is irreversible.

II. Activation-Induced Growth Inhibition as a Means of Treating T-Cell Tumors In Vivo

We have previously shown in a murine model system that activation of T-cell hybridoma tumors in vivo with the appropriate antigen can result in their elimination. The mechanism of elimination is two-fold: (1) a direct cytotoxic effect upon the T-cell tumors (see above), and (2) the induction of a host immune response. The latter has been investigated in a number of ways. The specificity of the mechanisms responsible for immunity to subsequent challenge with the same tumor was analyzed by rechallenging "cured" mice with a variety of syngeneic tumor cells. It was determined that these mice were able to reject the original (2B4.11) as well as other T-cell hybridomas, and the fusion parent, BW5147. However, two independently-derived AKR thymomas were not rejected. To determine if rejection could be due to the generation of cytotoxic cells, spleen cells from "cured" mice were removed and cultured in the presence of irradiated tumor stimulators. Cytotoxic activity was generated against the 2B4.11 cell but not against BW5147 or the AKR-derived thymomas. No NK or LAK activity was noted under these conditions. Finally, two in vivo approaches were used to determine which immune cells are responsible for the tumor rejection. Splenocytes that were adoptively transferred from "cured" animals to lightly irradiated recipients provided protection when the latter were challenged with fresh tumor cells. Depletion of Thy-1-bearing cells from the splenocytes abrogated this protection. Alternatively, T-cell subsets were depleted in vivo by the injection of specific mAbs. Initial studies suggest that the depletion of CD4+ cells has little effect on the ability of animals to reject the tumor when treated with antigen. However, animals depleted of CD8+ cells (typically cytotoxic T cells) were unable to reject the T-cell tumor even when treated with specific antigen. Together, these studies indicate that treatment of T-cell tumor-bearing animals with specific antigen results in the generation of cytotoxic T cells that play a critical role in the ultimate elimination of the tumor cells.

III. Radiation Sensitivity of Antigen Presentation

Resting B cells can present soluble antigen to T cells. Their ability to do so, however, is abolished by relatively high doses ($\geq$ 2000 rad) of gamma radiation. Despite reports by others that radiation exerts its negative effects at the level of antigen processing, our experiments have indicated that radiation mainly damages the ability of resting B cells to provide poorly-defined "costimulatory" signals. These two possibilities are difficult to separate, however, because the assays used to measure antigen presentation, typically lymphokine release or T-cell proliferation, require both of these APC properties to be intact. Therefore, we have used phosphatidylinositol hydrolysis as a rapid and quantitative measure of T cell responses to antigen. Phosphatidylinositol hydrolysis occurs within minutes of antigen recognition, plateaus at about 1 hr, and does not require the provision of costimulatory signals. Highly purified resting B cells were treated with up to 5000 rad and used to present the antigen pigeon cytochrome c fragment 81-104 (to avoid any requirements for processing) to two different antigen-specific T-cell clones. Measurement of phosphatidylinositol hydrolysis 1, 3, or 5 hr after stimulation with a fixed concentration of antigen found that unirradiated B cells and B cells given 1000 rad were equivalent. The delivery of 3000 or 5000 rad diminished the phosphatidylinositol hydrolysis in the responding T cell, but only by about 50%. Measuring phosphatidylinositol turnover 1 hr after stimulation with varying amounts of antigen confirmed this result, demonstrating about a 50% reduction in the maximal amount of phosphatidylinositol hydrolysis and a two-fold shift in the normalized dose-response curves. Although these studies do indicate that radiation has a deleterious effect on T-cell receptor occupancy, it appears to be minor compared to the dramatic effects it has on T-cell proliferation (generally $\geq$ 30-fold shift in dose-response curves). These data support the hypothesis that the major effect of radiation on antigen presentation is not at the level of providing a ligand for the T-cell receptor. What the exact nature of the costimulatory signal is, and why it is radiosensitive, is a subject for future experimentation.

SIGNIFICANCE

The finding that activation-associated growth inhibition is a two step process, each with a different Ca^{2+} requirement and susceptibility to CSA, indicates that there is multiple levels at which this phenomenon may be controlled. An understanding of the activation-initiated biochemical events that lead to growth inhibition of transformed T cells may increase our knowledge of what contributes to the neoplastic change in these cells. Furthermore, this "activation event" can complement lymphokine production in the study of the intracellular pathways that comprise cellular activation. Finally, in regard to the potential use of activation as a means of treating neoplasms in vivo, information about both the tumor's and the host's response to stimulation are essential if this phenomenon is to be extended to other tumor systems.

PUBLICATIONS

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Ashwell JD, Fox BS, Schwartz RH. T cell recognition of antigen and Ia molecules involves a trimolecular complex. In: Pernis B, Silverstein SC, Vogel HJ, eds. Processing and Presentation of Antigens. Orlando: Academic Press, 1988;273-90.

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Jenkins MK, Ashwell JD, Schwartz RH. Allogeneic non-T spleen cells restore the responsiveness of normal T cell clones stimulated with antigen and chemically-modified antigen-presenting cells, J Immunol 1988;140:3324-30.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09309-02 OAD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Activation Requirements and Diversity of $\gamma\delta$ T Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. M. Kruisbeek	Visiting Scientist	OAD, NCI
Others:	S. Marusic	Visiting Fellow	OAD, NCI
	M. A. Weston	Biologist	OAD, NCI
	L. Tentori	Visiting Fellow	OAD, NCI

COOPERATING UNITS *(if any)*

Laboratory of Immunology, NIAID (R. N. Germain); Massachusetts Institute of Technology (D. H. Raulet)

LAB/BRANCH

Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	1.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Antigen-specific murine and human T cells express a polymorphic receptor, consisting of two disulfide linked chains, termed α and β, noncovalently linked to a nonpolymorphic complex of proteins, termed T3. The T3 complex plays a vital role in the signal transduction process of T cells. Over the past year, a new type of T cell was discovered, expressing a T3 complex linked to two new chains, termed γ and δ. These cells express a double negative (CD4-CD8-) phenotype and are predominantly expressed in early T cell populations. To achieve a better understanding of the expression of this new receptor at the clonal level, panels of T-cell hybridomas with $\gamma\delta$ receptors were established from a variety of sources, i.e., adult thymus, fetal thymus, nude spleen, and nude spleen after thymus transplantation. Biochemical analysis of some of the T-cell hybridomas has demonstrated so far three different species of γ chain and four different species of δ chain, thus revealing a diversity in both γ and δ not expressed at the total population level. Furthermore, variable region gene usage for both the δ and the γ chain appears quite different for $\gamma\delta$ receptors on cells derived from different sources, and nonrandom pairing of γ and δ chains was observed. The study is aimed at acquiring a complete picture of the diversity of the expressed $\gamma\delta$ repertoire, which will involve a combination of biochemical and molecular analysis of the established cell lines. Future studies also include development of monoclonal antibodies specific for γ and δ chains, such that in vivo and in vitro studies on the effect of such antibodies on $\gamma\delta$ cells and their role in T-cell development can be explored. The significance of this project lies in the understanding the role of $\gamma\delta$ cells in early T-cell development.

PROJECT DESCRIPTION

PERSONNEL

Ada M. Kruisbeek	Visiting Scientist	OAD, NCI
Suzana Marusic	Visiting Fellow	OAD, NCI
Margaret A. Weston	Biologist	OAD, NCI
Lucio Tentori	Visting Fellow	OAD, NCI

OBJECTIVES

1. A study expression of the T_3-γ-δ-TCR-complex at the clonal level, to determine what signals activate $\gamma\delta$ cells, and to compare the spectrum of functional activities expressed by these cells with those of conventional $\alpha\beta$ cells;

2. To develop clones and hybridomas of antigen-specific T_3-γ-δ cells;

3. To develop monoclonal antibodies against γ and δ proteins, which will be used to explore the effect in vivo and in vitro elimination of cells expressing the $\gamma\delta$ receptor; and

4. To understand the physiologic role of T_3-γ-δ cells in the developing thymus and in the peripheral lymphoid tissues.

MAJOR FINDINGS

Since the recent identification of the protein products of the γ gene family on a new type of T cells in both mouse and man, much has been learned about their expression. The γ chain is, on most γ-expressing T cells, associated with a partner chain, termed δ. The $\gamma\delta$ heterodimer is associated with an invariant T_3-complex and appears predominantly, if not exclusively, on cells with CD4-CD8-phenotype both in the thymus and the periphery. Knowledge of the activation properties and potential diversity of T_3-$\gamma\delta$ cells, are essential to our understanding of the physiologic role of this distinct T-cell lineage. We have examined the T_3 components, activation properties, lymphokine production, and $\gamma\delta$ chain diversity of a panel of cloned T_3-$\gamma\delta$ T-cell hybridomas. Our results demonstrate that, by both functional and biochemical criteria, activation through the T_3-$\gamma\delta$ complex isolated from these hybridomas is currently used to raise monoclonal antibodies. These antibodies will be used to explore the effect of in vivo and in vitro elimination of cells expressing the $\gamma\delta$ complex on normal T-cell development.

PUBLICATIONS

Koning F, Kruisbeek AM, Maloy WL, Marusic-Galesic S, Pardoll DM, Shevach EM, Stingl G, Valas R, Yokoyama WM, Coligan JE. T cell receptor $\gamma\delta$ chain diversity. J Exp Med 1988;167:676-81.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09310-02 OAD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Early T Cell Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.M. Kruisbeek	Visiting Scientist	OAD, NCI
Others:	L. Tentori	Visiting Fellow	OAD, NCI
	M.A. Weston	Biologist	OAD, NCI
	J. Zuniga-Pflucker	General Fellowship Program	OAD, NCI

COOPERATING UNITS *(if any)*

Laboratory of Immunology, NIAID (W. E. Paul)

LAB/BRANCH

Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.6	0.8	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The signals and growth factors required for the development of T cells are largely unknown. During early fetal thymus ontogeny, a variety of cell surface molecules are expressed which could potentially serve as transducers of activation signals. Two such molecules (i.e., Thy-1 and the epsilon-component of the T3 complex) were explored and shown to function in early fetal thymocyte activation, as evidenced by both induction of proliferation and elaboration of lymphokines. We found that both IL-2 and BSF-1/IL-4 are produced upon activation of fetal thymocytes through Thy-1 and T3-activation, and are currently exploring, in an in vitro organ culture system, the effect of blocking IL-2 or IL-4 usage on T-cell development. These, as well as other lymphokines currently investigated, could play a role not only as growth factors, but, as preliminary studies indicated, also as differentiation factors. It appears that monoclonal antibody against IL-2 or IL-2 receptors block the development of $\alpha\beta$-TCR-bearing cells, while having no effect on $\gamma\delta$ cells. Antibodies against IL-4 had no effect, but their negative result requires further analysis.

PROJECT DESCRIPTION

PERSONNEL

Ada M. Kruisbeek	Visiting Scientist	OAD, NCI
Lucio Tentori	Visiting Fellow	OAD, NCI
Margaret A. Weston	Biologist	OAD, NCI
Juan Zuniga-Pflucker	General Fellowship Program	OAD, NCI

OBJECTIVES

1. To determine what signals determine intrathymic differentiation of T cells during fetal ontogeny, i.e., which receptor-ligand interactions and lymphokines are utilized as signals for expansion and differentiation of fetal thymocytes; and

2. To explore in vivo and in vitro models in the effects of blocking IL-2 and IL-4 usage on T-cell development.

MAJOR FINDINGS

The cellular and humoral factors required for intrathymic growth and differentiation of fetal T cells are largely unknown. We recently found that early fetal thymocytes can utilize both the Thy-1 molecule and the T3-complex as transducers of activation signals. In addition, we demonstrated that fetal thymocytes produce both IL-2 and IL-4 upon activation. As these studies were performed at a timepoint before conventional $\alpha\beta$ receptor bearing cells are present, the results suggest a role for early $\gamma\delta$ receptor bearing cells in development, i.e., production of lymphokines. Current studies focus on: (1) which other cell surface molecules are involved in the delivery of successful activation signals to early T cells; and (2) which role the $\gamma\delta$ cells play in the development of $\alpha\beta$ cells.

PUBLICATIONS

Tentori L, Pardoll DM, Zuniga JC, Hu Li J, Paul WE, Bluestone JA, Kruisbeek AM. Induction of proliferation and interleukin 2 and 4 production in early fetal thymocytes by activation through Thy-1 and CD3, J Immunol 1988;140:1089-94.

Fowlkes BJ, Kruisbeek AM, That HT, Weston MA, Coligan JE, Schwartz RH, Pardoll DM. A novel population of T-cell receptor bearing thymocytes, Nature 1987;329:251.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09311-02 OAD

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen-Specific Receptor Structure and Function in T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. D. Ashwell	Senior Investigator	OAD, NCI
Others:	M. Mercep	Visiting Fellow	OAD, NCI

COOPERATING UNITS *(if any)*

Laboratory of Cell Biology and Metabolism, NICHHD (J. Bonifacino, L. Samelson, and R. Klausner), Laboratory of Immunology, NIAID (T. Saito and R. Germain)

LAB/BRANCH

Office of the Associate Director

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To analyze the contribution of individual T-cell antigen receptor (TCR) components to the structure and function of the complete complex, variants of an antigen-specific T-cell hybridoma that survived TCR-mediated stimulation in vivo were cloned and grown in vitro. In addition to cells that had lost the CD3-ζ chain, examples of which we have previously described, clones were isolated that had very low levels of the CD3-$\zeta\eta$ heterodimer, a pairing that is present in approximately 20% of the TCR complexes of a given cell. Whereas stimulation of these clones with antigen or antibodies to the TCR yielded normal levels of tyrosine kinase activation in these cells, the levels of phosphatidylinositol hydrolysis were dramatically reduced. Comparison of the wild type T-cell hybridoma with six different variants yielded a remarkably good positive correlation between CD3-$\zeta\eta$ expression and the ability to generate inositol phosphates upon stimulation. These results have been interpreted to indicate that TCR's that contain the CD3-$\zeta\eta$ heterodimer interact with a phospholipase C, leading to the hydrolysis of phosphatidylinositol. TCR's that do not contain CD3-$\zeta\eta$ (i.e., contain a CD3-ζ2 homodimer) interact with a tyrosine kinase pathway. These data suggest a novel biological mechanism whereby a single ligand-binding molecule can initiate independent intracellular pathways due to an association with different signal transducing elements.

PROJECT DESCRIPTION

PERSONNEL

Jonathan D. Ashwell	Senior Investigator	OAD, NCI
Mladen Mercep	Visiting Fellow	OAD, NCI

OBJECTIVES

(1) To analyze the structural and functional roles of the different components of the T-cell antigen-specific receptor;

(2) To determine which second signals, such as phosphatidylinositol metabolism or increases in intracellular free Ca^{2+}, are involved in T-cell activation; and

(3) To distinguish, if possible, those signals that lead to lymphokine production and those that lead to the death of transformed T cells.

MAJOR FINDINGS

The T-cell antigen-specific receptor (TCR) has two major subunits: the antigen-binding heterodimer (Ti) composed of the α and β chains, and the CD3 complex, composed of the γ, δ, ϵ, ζ, and η (formerly p21) chains. Metabolic and extrinsic labeling studies have shown that the CD3-ζ chain exists as a disulfide-linked dimer. Furthermore, each TCR-bearing T cell has two structurally different CD3 complexes, one (comprising ~80% of TCR's) that has a ζ chain homodimer (CD3-ζ_2), and another that has a ζ chain disulfide-linked to an η chain (CD3-$\zeta\eta$). We have previously described T-cell TCR variants that have lost either the CD3-ζ, CD3-γ or Ti-β chain. In an attempt to produce other types of mutants, two mice were inoculated with the antigen-specific T-cell hybridoma, 2B4.11, and treated with the anti-CD3-ϵ mAb, 2C11. Tumors grew in both mice. The tumors were excised and the cells cloned by limiting dilution. All the clones derived from one of the tumors had lost the CD3-ζ chain and were phenotypically identical to the CD3-ζ loss variant previously described. Most of the clones from the other tumor had normal levels of CD3-ζ, but approximately one-third were deficient in CD3-$\zeta\eta$. Two independently-derived subclones, EV.3 and 2M.44, were chosen for further study. Immunoblotting and densitometric analysis demonstrated that in 2B4.11, 11% of the ζ chain is covalently bound to η. In EV.3 and 2M.44, this value is 0.8% and 1.5%, respectively. All three cells bore relatively normal levels of TCR on the cell surface, with EV.3 being consistently ~33% lower than 2B4.11. To study the consequences of the loss of CD3-$\zeta\eta$, the early biochemical response of phosphatidylinositol hydrolysis was measured. In response to stimulation with antigen or anti-TCR mAbs, 2B4.11 rapidly generates large amounts of water-soluble inositol phosphates. In contrast, both EV.3 and 2M.44 were deficient in this regard. Another characteristic early biochemical change upon stimulation, increases in intracellular Ca^{2+}, was also markedly decreases in these cells compared to the wild type. Phosphatidylinositol hydrolysis, and the concomitant generation of diacylglycerol, is thought to be primarily responsible for the activation of protein kinase C. Reasoning that the activation of this enzyme should also be deficient in EV.3 and 2M.44 cells, the serine phosphorylation of CD3-γ, which has been shown to be mediated by protein kinase C, was measured. It, too, was found to be substantially diminished. In contrast, the phosphorylation of CD3-ζ on a tyrosine residue was comparable between all cells. These results prompted us to reexamine a previously described variant of 2B4.11, T$\alpha\beta$1.2. Activated T$\alpha\beta$1.2 cells were independently found to generate little phosphatidylinositol hydrolysis or increases in intracellular Ca^{2+} despite excellent lymphokine production. Examination of these cells for CD3-$\zeta\eta$ found that, like EV.3 and 2M.44, they were deficient. In fact, a total of 6 2B4.11 subclones with different levels of CD3-$\zeta\eta$ were analyzed for their ability to generate inositol phosphates upon stimulation with either antigen or anti-CD3 mAbs. It was found that there was a strikingly linear and statistically significant correlation between these two attributes ($p < 0.05$). From these data we propose that the pathways utilized in T-cell transmembrane signaling depend upon the composition of the CD3 complex. One complex, containing CD3-ζ_2, activates a tyrosine-specific kinase. The other complex, containing CD3-$\zeta\eta$, activates a phospholipase C that, in turn, generates inositol phosphates and diacylglycerol. These data suggest a novel biological mechanism whereby a single ligand-binding molecule can initiate independent intracellular pathways due to an association with different signal transducing elements.

SIGNIFICANCE

Receptors at the plasma membrane are believed to initiate intracellular events via the generation of biochemical "second messengers". One of the paramount questions about this event is how does receptor occupancy translate into signal generation? Because of the ability to raise mutants and variants that have lost specific chains, the structural complexity of the TCR has, paradoxically, proved to be an asset in this regard. The present finding that CD3-$\zeta\eta$ appears to be necessary for TCR-mediated phosphatidylinositol hydrolysis is a step toward an understanding of how this multi-unit structure effects cellular responses.

PUBLICATIONS

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Bonifacino JS, Chen C, Lippincott-Schwartz J, Ashwell JD, Klausner RD. Subunit interactions within the T cell antigen receptor: clues from the study of partial complexes, Proc Natl Acad Sci USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09323-01 OAD

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Selection of the T Cell Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. M. Kruisbeek	Visiting Scientist	OAD, NCI
Others:	S. Marusic	Visiting Fellow	OAD, NCI
	M. A. Weston	Biologist	OAD, NCI

COOPERATING UNITS *(if any)*
Experimental Immunology Branch, NCI (A. Singer)

LAB/BRANCH
Office of the Associate Director

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Differentiation of bone marrow derived precursors into mature T cells takes place in the thymus. During differentiation, T cells develop the receptor repertoire which allows them to recognize antigen in the context of self major histocompatibility complex (MHC) molecules. Mature T helper cells (mostly CD4+CD8-) recognize antigen in the context of class II MHC molecules, whereas cytotoxic T cells (mostly CD4-CD8+) recognize antigen in the context of class I MHC determinants. Thymic MHC-encoded determinants greatly influence the selection of the T-cell receptor repertoire. In addition to positive selection, a negative selection to eliminate self-reactive T-cell clones is thought to occur in the thymus, but how this "education" occurs is not well understood. It has been suggested that during differentiation an interaction between the T-cell receptor (TCR) and MHC-encoded determinants occurs, leading to the selection of an MHC-restricted receptor repertoire. In support of this hypothesis, class-II-specific, CD4+CD8- helper T cells fail to develop in mice neonatally treated with anti-class II monoclonal antibody (our earlier work). Our current work addresses whether the same rules apply to development of CD4-CD8+ T cells. Furthermore, the possibility that the selection of the T cell repertoire is influenced by interactions other than those between TCR's and MHC, by analyzing the role of the CD4 and CD8 molecules in development. Recent results demonstrate that, indeed, also CD4-CD8+ T cells require MHC expression for their development to occur. The significance of this project lies in: (1) understanding the factors that control development of T cells; and (2) applying this knowledge to studies on tolerance induction, which is greatly influenced by CD4- expression.

PROJECT DESCRIPTION

PERSONNEL

Ada Kruisbeek	Visiting Scientist	OAD, NCI
Suzana Marusic	Visiting Fellow	OAD, NCI
Margaret A. Weston	Biologist	OAD, NCI

OBJECTIVES

To determine the role of CD4, CD8, TCR and MHC antigens during T-cell development.

MAJOR FINDINGS

Blocking of expression of MHC antigens leads to a failure to develop mature T cells. Current studies focus on the cause of this phenomenon, i.e., is it due to blocking a positive signal delivered through TCR-MHC interactions or through accessory molecule-MHC interactions or both.

PUBLICATIONS

Kruisbeek AM, Zuniga-Pflucker J, Marusic-Galesic S, Weston MA, Tentori L, Longo DL. Thymic selection of the T cell repertoire, Immunol Res, in press.

Marusic S, Stephany DA, Longo DL, Kruisbeek AM. Development of CD4-CD8+ cytotoxic T cells requires interactions with class I MHC determinants, Nature 1988;333:180-3.

Mizuochi T, Tentori L, Sharrow SO, Kruisbeek AM, Singer A. Differentiation of Ia-reactive CD8+ murine T cells does not require Ia engagement, J Exp Med, in press.

SUMMARY REPORT

BIOLOGICAL RESOURCES BRANCH

October 1, 1987 To September 30, 1988

INTRODUCTION

The Biological Resources Branch (BRB) is the extramural arm of the Biological Response Modifiers Program (BRMP).

The BRB supports preclinical and clinical biological response modifiers (BRMs) research in the biomedical community through a program of grants and contracts. The Branch sponsors Phase I and early Phase II clinical studies which assess biological effects of BRMs in patients and correlate changes in the biological responses with antitumor activity. A Preclinical Evaluation Laboratory performs in vivo modeling and efficacy assessments of BRMs in animal models. A repository distributes selected agents for preclinical studies. Other contracts support the production and in vivo evaluation of monoclonal antibodies, immunoconjugates and other BRMs.

OFFICE OF THE CHIEF

Chief, BRB	Stephen P. Creekmore, Ph.D., M.D.
Program Analyst	Alfred C. Denn
Secretary	Virginia L. Axline
Clerk-Typist	Dawn M. Troxell

PRECLINICAL STUDIES GROUP

Ira Green, M.D.
Toby T. Hecht, Ph.D.
Craig W. Reynolds, Ph.D.

CLINICAL TRIALS GROUP

Stephen P. Creekmore, Ph.D., M.D.
Carl M. Pinsky, M.D.
Michael B. Stewart, M.D.

BIOLOGICAL RESOURCES BRANCH, PRECLINICAL STUDIES GROUP

This group is responsible for monitoring the results of BRMP-supported preclinical research as well as testing, production and development contracts supported by the BRMP. Project Officers on all BRB contracts in the preclinical area are a member of this group, as are the Program Directors for all grants in the BRB. This group also identifies and acquires BRMs of interest to the BRMP through its relationships with extramural laboratories and biotechnology companies. Relevant information on potential agents is directed toward appropriate program personnel and working groups, such as the BRMP Operating Committee (OC), BRMP-CTEP Working Group (BCWG), and the BRMP-DTP Working Group (BDWG). The Preclinical Studies Group also directs a contract program for the development of BRMs through screening of potential agents, animal testing and therapeutic trials, and serves as a liaison for this activity to the Developmental Therapeutics Program. Other responsibilities include the development of appropriate experimental systems for detection and evaluation of potential BRMs and the coordination, planning and monitoring of detailed evaluations of BRMs in relevant systems.

BIOLOGICAL RESOURCES BRANCH, CLINICAL TRIALS GROUP

The BRB Clinical Trials Group initiates and monitors Phase Ia and Ib clinical trials of BRMs through contracts, e.g., Master Agreement Orders. An important object of these trials is the correlation between changes in immunological reactivity and clinical efficacy and toxicity in these studies. This group maintains close liaison with the Investigational Drug Branch and the Regulatory Affairs Branch of the the Cancer Therapy Evaluation Program (CTEP) of the Division of Cancer Treatment, to coordinate appropriate development of investigational agents for clinical trials, and for regulatory issues.

Monitoring of Phase II clinical trials with CTEP personnel is an ongoing arrangement in which the BRMP and CTEP work jointly, through the BRMP-CTEP Working Group (BCWG).

This Group also coordinates efforts with CTEP in the planning and review of Phase III clinical trials which include BRMs.

SUMMARY OF FY 88 ACTIVITIES

During this year the BRB has been involved in the following:

- Received grant applications as a result of previously issued Program Announcements. Seven awards were made as a result of the Announcements.
- Grant application for 3 new Requests for Applications (RFAs) were reviewed. Forty-seven applications were received. Four grants are to be funded.
- Re-evaluated and restructured the contract system for preclinical evaluation of BRMs.
- Maintained 29 contracts for testing BRMs in Phase I clinical trials.
- Awarded 5 new clinical Task Order Contracts in FY 88 under Master Agreements held by 14 institutions.

- Initiated 15 new Phase I clinical protocol studies under the Master Agreement contracts.
- Evaluated 7 compounds in the BRMP Preclinical Evaluation Laboratory.
- Continued distribution of interleukin-2 (IL-2) Reference Standard to the scientific community worldwide.
- Recommended 8 Phase I with 1 awarded and 2 Phase II SBIR contracts which were awarded.
- Reviewed 30 proposals for new 5-year Phase I clinical contracts, selecting 23 for further negotiations potentially leading to contract awards.

GRANTS AND CONTRACT ADMINISTRATION

The BRB provides planning, direction, implementation, and evaluation of research supported by grants and contracts. The Branch staff maintains liaison with all pertinent peer review groups involved in grant and contract review.

Liaisons have been established and maintained with other programs in the NCI, including the Immunology and Tumor Biology Programs in the Division of Cancer Biology and Diagnosis (DCBD), to minimize overlap in the grant and contract areas. A regular working relationship is maintained with the Developmental Therapeutics Program (DTP) of the Division of Cancer Treatment (DCT) to coordinate drug and biologic development. A cooperative clinical protocol evaluation system with the CTEP, DCT has been established to coordinate Phase I, Phase II, and Phase III BRM Clinical Trials. This includes membership of several staff members of CTEP on the BRM Decision Network Committee and the establishment of a BCWG.

ACCOMPLISHMENTS OF BRMP GRANTS PROGRAM FY 88

A total of 101 grants are supported by the BRMP in FY 88. The total dollar amount for these grants was $19.8 million. Of these, 10 were Program Projects and 73 were R01 grants, the remainder being Outstanding Investigator Award (1), Merit Awards (6), New Investigator Awards (3), Conference Grants (2), AREA grant (1), and SBIR grants (5). A total of 207 competing grant applications were received in FY 88, 39 of which were funded. Fifteen of these grant applications were received in response to BRMP Program Announcements, of which 7 were funded. Table I is a complete listing of all BRMP grants, classified by primary scientific thrust.

About one-third of our current grants address the topic of the development and/or use of monoclonal antibodies in cancer therapy. A number of these grants are investigating the therapy of malignant melanoma, a disease which is particularly resistant to most forms of treatment. The development and characterization of monoclonal antibodies reactive against melanoma antigens and their use to prevent invasion and metastases of human melanoma in an animal model is supported by an NCI Outstanding Investigator Award. The finding in this project that anti-ganglioside monoclonal antibodies of the IgG3 isotype are highly effective in "arming" IL-2 stimulated LAK cells to kill human tumors in nude mice is a preclinical observation which may be clinically useful. A program project is funded in which anti-ganglioside monoclonal antibodies developed in the laboratory have been used in clinical trials with melanoma patients with a number of clinical responses being observed. Of particular interest in another grant-supported clinical trial was the finding in one laboratory that intralesional injection of human monoclonal antibody resulted in a number of promising clinical responses in melanoma patients.

Considerable effort was also expended in the grant program this year to develop new methods or the extension of existing methods of conjugating toxins or radiolabelled compounds to specific monoclonal antibodies to improve radioimaging and therapy. Particularly innovative studies this year included the development of methods to conjugate high levels of 10-boron to monoclonal antibodies by the synthesis of new carborane compounds, the ultimate aim being to use these 10-boron labelled antibodies for neutron capture therapy of tumors.

A 1-day conference, jointly sponsored by the BRMP and the DTP, on the topic of "Specific Cancer Cell Targeting Using Molecular Genetic Technology" was held on June 1, 1988. Fifty participants discussed data, ideas and problems associated with this exciting new area for cancer therapy.

A second 3-day conference which will be sponsored by the AIDS Program (NIAID), the BRMP, and the NIEHS, entitled "Development of Cytokines for the Treatment of AIDS," will be held on July 31 - August 3, 1988. One hundred-twenty participants are expected.

Several grants were concerned with the use of liposomes. In some studies, liposomes were conjugated with specific monoclonal antibodies, to attempt to increase the targeting of drugs or lymphokines. Several of these grants directly address the problem of high reticuloendothelial uptake of liposomes, a phenomenon which results in inefficient drug delivery to the tumor. Liposomes of different chemical composition have been designed and it has been shown that some of these have a very greatly decreased liver uptake and increased serum half-life, holding out promise for more effective therapy. Of particular potential benefit are studies which are being carried out to investigate factors which affect the intracellular fate and the release of liposome encapsulated drugs from liposomes of different chemical compositions.

More than 15 grants are current in the area of lymphokine therapy for cancer. Both preclinical and clinical studies of the efficacy of IL-2 or IL-2 and LAK cells are in progress. Preclinical studies on the nature of B-cell activating and differentiating factors should yield information of ultimate clinical benefit. A new approach to therapy of cancer with autologous leukocytes uses cultured host cells which have been prepared from the patient's own tumor. This approach is being used in preclinical models, and a clinical trial of these cells as therapy of lung cancer was initiated this year.

In the area of molecular biology, the tumor cell biology of the fms oncogene proteins is being investigated. These are of particular interest to this program, since the c-fms protein appears to be the receptor for the macrophage growth factor, CSF-1. The murine c-fms gene has been cloned, and the molecular changes that activate the c-fms proto-oncogene are under investigation. The production of monoclonal antibodies to the cloned external domain of the c-fms protein is planned and will be of particular interest as a potential therapeutic agent. Novel approaches include the production of engineered diphtheria toxin-peptide hormone molecules, the introduction into cells of an engineered toxin gene to facilitate selective cell killing, and the use of anti-sense RNA.

A program project on AIDS research is current, which is investigating a number of preclinical and clinical aspects of AIDS. The identification of an anti-HIV activity of the agent castanospermine, which alters viral glycoproteins and blocks replication and infection by HIV was partially funded by one of the BRMP grants.

We also anticipate further developments from the currently funded grants, including results from new clinical trials of monoclonal antibodies and of therapy with autologous leukocytes.

Request for Applications

In FY 87 the BRMP issued several new RFAs: Manipulation of the Suppressor Arm of the Immune Response Directed Towards Successful Human Immunotherapy; Protection of Bone Marrow Against the Effects of Cytotoxic Drugs and X-Irradiation; and Immunologic Investigation of Multi Drug Resistance of Neoplastic Cells. A total of 47 applications were approved for funding. Four will be funded based upon the payline or exception to the funding plan. In addition, one award from the RFA issued in FY 85 "Use of Oncogene Related Products for Cancer Therapy" is continuing into this year.

Program Announcements

In FY 86 the BRMP issued six Program Announcements: Development of New Methods to Couple Cytotoxic Agents to Monoclonal Antibodies; Preclinical Studies of LAK Phenomenon; Determination of the Therapeutic Usefulness of Purified Cytokines in Cancer Models; Use of Oncogene Related Products for Cancer Therapy; Application of Neuroendocrine Effects on the Immune System for Cancer Therapy; and Determination of the Therapeutic Usefulness of Maturation, Differentiation and Anti-Growth Factor Substances in Cancer Models. Grant applications are still being received for these Program Announcements and six others issued in the past. There have been 326 grant applications received to date and 55 of these have been funded.

CONTRACT PROGRAM

The Branch staff initiates Requests For Proposals and provides programmatic direction, evaluation and monitoring for contracts supported by the BRMP. During FY 88, the BRB awarded approximately $5.57 million for 18 extramural contracts, including $2.9 million in clinical task order contracts and $2.37 million in 7 non-clinical contract awards: 8 were new awards and 2 received incremental funding. The contracts active and projected for funding in FY 88 are shown in Table II. Contracts currently in the BRMP include those related to collection, storage, quality assurance; clinical development and distribution of BRMs; chemical coupling of cytotoxic agents to MoAb; and procurement of BRMs for preclinical and clinical testing.

In addition, the BRB has been actively involved in the Small Business Innovative Research (SBIR) program of the NIH. Active SBIR topics currently include the following: Monoclonal Antibody Development for Clinically Relevant Research; Development and Characterization of Oncogene-Related Products for Therapy;

Monoclonal Antibody Conjugates for Therapy and Diagnosis; Production of Liposomes with Biological Response Modifiers and Medicinals for Therapy; Production of Tumor Cell Lines Sensitive to Specific Cytokines; and Data Bank on MoAbs for Clinical Cancer Therapy and Diagnosis. There are 4 current Phase II contracts awarded in the area of monoclonal antibody conjugation technology and 1 Phase I contract for a monoclonal antibody data bank.

PHARMACEUTICAL COMPANY RELATIONSHIPS

The BRMP has established relationships with most of the biotechnology and pharmaceutical companies which produce BRMs. Relationships exist with Burroughs-Wellcome, Hoffmann-LaRoche, Schering, Biogen, Cetus-Shell, Genentech, Collaborative Research, Hybritech, AMGEN, Immunex, Becton Dickenson, Hoechst-Roussel, Dainippon, Sandoz Research Institute, Ortho, Brunswick, Cell Tech, Lederle (American Cyanamid), Eastman, Auire, etc. Each of these companies is currently supplying agents for clinical trials. A number of other companies have supplied compounds for preclinical evaluation. In some cases, clinical trials evaluating these new compounds are currently being planned.

The BRB is currently expanding its relationships with the Biotechnology Industry by offering the opportunity to collaborate on joint Government:Industry Programs for those BRMs with the greatest potential for clinical utility.

NEW INITIATIVES

In addition to the new initiatives already noted in the preceding text, the following areas are being developed:

- A Master Agreement mechanism for procurement of products and services has been approved by the DCT Board of Scientific Advisors.

- Establishment of full-time, Phase I clinical contractors to provide a more stable base for investigations of monoclonal antibodies and cytokines.

- Establishment of a Master Agreement mechanism for clinical trials involving complex or sophisticated immunological manipulations for the treatment of cancer or AIDS.

PRECLINICAL EVALUATION LABORATORY

The Preclinical Evaluation Laboratory (PEL) has concentrated on several areas during the first half of FY 88. These include the area of bone marrow protection and restoration and the role of chemoimmunotherapy in the treatment of metastatic cancer in mice. The laboratory has confirmed and extended the observation that interleukin-1 (IL-1) has a profound protective effect on the bone marrow of mice when given 20 hours prior to a lethal dose of irradiation. The protective effect correlated with preservation of colony forming units (CFU-C) in the bone marrow as well as increased peripheral blood mononuclear cell counts. IL-1 alone, when administered prior to radiation, appears to be completely responsible for the protective effects since additional IL-1 administered after radiation or use of granulocyte-macrophage colony stimulating factor (GM-CSF) in combination with IL-1 did not enhance its protective effects.

The PEL continued its work to determine the optimal dose, route and schedule of administration of both G- and GM-CSF in mice receiving the cytotoxic agent cyclophosphamide. Experiments have been initiated to determine the effects of the CSFs on the ability of animals to endure multiple cycles of chemotherapy. The laboratory has experiments in progress to detect the possibility of stem cell exhaustion in mice receiving treatment with CSFs. Toward that end, dose response toxicity studies are being performed in mice with melphalan and doxorubicin to determine the effects of CSFs on mice after treatment with chemotherapeutic regimens with varying toxicity for the stem cell population. Experiments have also been undertaken in tumor-bearing mice to determine the effects of scheduling on the restorative capabilities of GM-CSF when used with multiple injections of cyclophosphamide. Preliminary results indicate that scheduling a second dose of a chemotherapeutic agent following restorative therapy with GM-CSF is a critical factor in the successful application of this antitumor strategy. The administration of a second cyclophosphamide dose 10 days after completion of restorative therapy yielded the lowest PBL count nadir, the most prolonged bone marrow restorative effect (as determined by measuring colony forming units), and a significant and meaningful increase in survival duration (the early nature of the experiment precludes the determination of long-term survival). Based on these data, dose response experiments to optimize the antitumor and bone marrow restorative responses are in progress.

The development of a preclinical model for bone marrow restoration now allows for a rational approach to determine the long-term effects of CSFs on the ability of animals to endure multiple cycles of chemotherapy as well as determine the possibility of stem cell exhaustion in mice receiving a variety of chemotherapeutic regimens. Preliminary dose response toxicity studies are being performed in mice with other clinically useful chemotherapeutic drugs to determine the effects of CSFs after treatment with chemotherapeutic regimens with varying toxicity for the stem cell population.

Another factor, colony stimulating factor-1 (M-CSF) has been obtained from Genetics Institute and early experiments have examined the dose response and kinetics of M-CSF on mouse peripheral blood mononuclear cells, bone marrow cellularity and colony forming ability. This agent is presently also being tested for antitumor effects in the B16 melanoma metastasis and spontaneous tumor models.

The PEL has been actively engaged in experiments designed to incorporate various biological agents into chemotherapy regimens in order to develop chemoimmunotherapy regimens with antitumor activity. The CSFs and IL-1 have marginal antitumor activity when used alone, but combinations of these agents with chemotherapy to debulk the tumor can provide additional therapeutic benefit. Recent studies have shown that recombinant cytokines can enhance the therapeutic activity of chemotherapy in metastatic disease. All the variables which determine the success of this approach have not yet been determined, but are currently under investigation. In view of the great promise of this chemoimmunotherapeutic approach, these studies will continue in the future.

Work on the bone marrow protective and restorative effects of the hematoregulatory peptides 5b (HP5b) monomer and dimer continues. HP5b, when in its monomeric form, has been shown to have bone marrow protective capabilities similar to IL-1 when administered prior to chemotherapy or radiotherapy. In the dimeric form, HP5b has also demonstrated a dose-dependent hemopoietic stimulating capacity, and also has shown radioprotective capabilities comparable with IL-1. Therefore, experiments are underway to utilize both the marrow protective and restorative activities (monomeric and dimeric forms respectively) of HP5b as a single regimen combined with a chemotherapeutic protocol in tumor-bearing mice.

Finally, the PEL has been actively engaged in experiments designed to incorporate various biological agents into chemotherapy regimens in order to develop chemoimmunotherapy regimens with antitumor activity. Until very recently, the clinical use of recombinant interleukin-2 (rIL-2) has been associated mostly in combination with LAK cell therapy. The PEL is now investigating an alternative strategy where rIL-2 is utilized in combination with the investigative drug flavone acetic acid (FAA), a chemoimmunotherapeutic combination which demonstrates synergistic antitumor activity against established, as well as metastatic murine renal cancer. The specificity of the antitumor response, and the effector mechanisms responsible for the antitumor response are currently under investigation. The determination of lymphokine induction by FAA and/or rIL-2, and the role this induction in the therapeutic efficacy of this regimen is being studied in collaboration with the laboratories of Drs. Robert Wiltrout and Howard Young, BRMP, DCT, NCI-FCRF.

BIOLOGICAL RESPONSE MODIFIERS PROGRAM OPERATING COMMITTEE (BRMPOC)

The BRMPOC acts as the steering committee for the BRMP extramural program. The BRMPOC reviews and prioritizes agents as to evaluation in the preclinical screens. The recommendations of the BRMPOC are subsequently presented to the DNC and in turn the recommendations of the DNC are implemented by the BRMPOC and program staff. Members of the BRMPOC are asked, on a rotating basis, to review and summarize data from pharmaceutical firms and present their recommendations on any candidate agent to the committee.

PUBLICATIONS

Creekmore SP, Longo DL. Biologic response modifiers: interferons, interleukins and other cytokines. Resident and Staff Phys. In Press.

Francis GE, Pinsky C. Growth and differentiation control. In: Pinedo HM, Longo DL, Chabner BA, eds. Cancer chemotherapy and biological response modifiers annual 9. Amsterdam: Elsevier, Science Publishers 1987;502-29.

Francis GE, Pinsky CM. Clinical trials of differentiation therapy: current trials and future prospects. In: Waxman S, Rossi GB, Takaku F, eds. The status of differentiation therapy of cancer. New York: Raven Press, 1988;331-47.

Gregg EO, Bridges SH, Youle RJ, Longo DL, Houston LL, Glennie MJ, Stevenson FK, Green I. Whole ricin and recombinant ricin A chain idiotype-specific immunotoxins for therapy of the guinea pig L2C B-cell leukemia. J Immunol 1987;138:4502.

Mason L, Giardina SL, Hecht T, Ortaldo J, Mathieson BJ. LGL-1: A non-polymorphioantigen expressed on a major population of mouse natural killer cells. J Immunol 1988;140:4403-12.

Messerschmidt GL, Henry DH, Synder HW JR, Bertram J, Mittelman A, Ainsworth S, Fiore J, Viola MV, Louie J, Ambinder E, MacKintosh FR, Higby DJ, O'Brien P, Kiprov D, Hamburger M, Balint JP Jr, Fisher LD, Perkins W, Pinsky CM, Jones FR. Protein A immunoadsorption in the treatment of malignant disease. J Clin Oncol 1988;6:203-12.

McMannis JD, Fisher RI, Creekmore SP, Braun DP, Harris JE, Ellis TM. In vivo effects of recombinant IL-2. I. Isolation of circulating Leu-19+ lymphokine-activated killer effector cellsfrom cancer patients receiving recombinant IL-2. J Immunol 1988 140:1335-40.

Peschel C, Green I, Ohara J, Paul WE. Role of B-cell stimulatory factor 1/interleukin 4 in clonal proliferation of B-cells. J Immunol 1987 139:3338-47.

Sayer TJ, Twilley TA, Bull CA, Denn III AC, Pilaro AM, Lokesh B. Effect of cytokines on polymorphonuclear neutophil (PMN) infiltration in the mouse: prostaglandin- and leukotriene-independent induction of infiltration by interleukin 1 and tumor necrosis factor. J Immunol In Press.

TABLE I

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
DEVELOPMENT AND/OR APPLICATION OF MONOCLONAL ANTIBODIES FOR CANCER THERAPY (Grants listed by type of tumor investigated)		
Leukemia - Preclinical		
CA18105	Applebaum, F. R.	"Immunotherapy Study With Spontaneous Malignancies"
CA39930	Bast, R. C.	"Specific Immunotherapy With Monoclonal Antibodies"
CA26386	Bernstein, I. D.	"Monoclonal Antibody Therapy of Cancer"
CA43735	Herlyn, D.	"Induction of Anti-Tumor Immunity by Anti-Idiotypes"
CA38845	Grant, C. K.	"Monoclonal Antibody Therapy of Feline Leukemia"
CA37131	Seon, B. K.	"Monoclonal Antibodies for Therapy of Cancer"
CA43350	Siegel, R. C.	"Immunoadsorption of Malignant Cells From Bone Marrow"
CA28149	Vittetta, E. S.	"New Strategies for Immunotoxin Therapy"
CA47334	Epstein, A. L.	"Immunotherapy of Human Lymphomas With Lym-1 and Lym-2"
Leukemia - Clinical		
CA31888	Ball, E. D.	"Monoclonal Antibodies for the Treatment of Acute Myelogenous Leukemia"
CA44991	Bernstein, I. D.	"Therapy of Leukemia/Lymphoma With Monoclonal Antibodies"
CA33399	Levy, R.	"Human Anti-Tumor Therapy With Monoclonal Antibodies"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
DEVELOPMENT AND/OR APPLICATION OF MONOCLONAL ANTIBODIES FOR CANCER THERAPY		
Leukemia - Clinical (Continued)		
CA37497	Royston, I.	"Monoclonal Antibodies in Cancer Detection and Treatment"
CA41081	Uhr, J. W.	"Immunotoxin Therapy for Patients With B Cell Tumors"
Lung Cancer - Preclinical		
CA33462	Bankert, R. B.	"Monoclonal Antibodies Applied to Treat/Diagnose Cancer"
Ovarian Cancer - Preclinical		
CA37646	Chu, T. M.	"Targeting and Therapy of Tumors With Monoclonal Antibody"
Brain Cancer - Preclinical		
CA29125	Gillespie, G. Y.	"Monoclonal Antibodies to Glioma Cells"
Mammary Tumor - Preclinical		
CA30924	Sirbasku, D. A.	"Monoclonal Antibodies to Mammary Tumor Growth Factors"
Colorectal Cancer - Preclinical		
CA30728	Griffin, T. W.	"Anti-CEA Immunotoxins"
CA31753	Hawthorne, M. F.	"10-Boron Labeled Antibodies in Cancer Therapy"
CA33470	Strand, M.	"Chemically Modified Monoclonal Antibody in Tumor Models"
Colorectal Cancer - Clinical		
CA44167	Sears, H. F.	"Monoclonal Antibody in Cancer Therapy"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
DEVELOPMENT AND/OR APPLICATION OF MONOCLONAL ANTIBODIES FOR CANCER THERAPY		
Melanoma - Preclinical		
CA38011	Hellstrom, I. E.	"Monoclonal Antibodies to Melanoma Associated Antigens"
Melanoma - Clinical		
CA42508	Reisfeld, R. A.	"Molecular Profile of Melanoma and Neuroblastoma Antigens"
CA33049	Oettgen, H. F.	"Monoclonal Antibodies in the Treatment of Cancer"
CA42396	Irie, R. F.	"Treatment of Melanoma With Human Monoclonal Antibody"
Prostate Cancer - Preclinical		
CA34536	Lee, Ching-Li	"Monoclonal Antibody in Treatment of Prostatic Cancer"
CA39690	Webb, K. S.	"Chemoimmuno(toxin) Therapy of Metastatic Prostate Carcinoma"
Anti-Receptor or Anti-Oncogene Product Monoclonal Antibody - Preclinical		
CA42060	Mendelsohn, J.	"Anti-Tumor Activities of Anti-Receptor Antibodies"
CA45187	Roth, J.	"Tumor Antigen Expressed by Oncogene Transformed Cells"
Hybrid & Chimeric Antibodies - Preclinical		
CA39870	Huston, J. S.	"Engineering of Antibody Binding Sites to Tumor Antigens"
CA45232	Khazaeli, M. B.	"Study of Mouse/Human Chimeric Monoclonal Antibodies"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
CYCLOPHOSPHAMIDE		
Clinical		
CA39248	Berd, D.	"Augmentation of Human Immunity By Cyclophosphamide"
Preclinical		
CA31545	Enker, W. E.	"Suppressor Cell Elimination As Immunotherapy"
MOLECULAR BIOLOGY		
Preclinical		
CA40892	Benjamin, T. L.	"Antisense RNA Regulation of Proto-Oncogene Expression"
CA42354	Maxwell, I. H.	"Tumor Cell Killing by Expression of a Toxin Gene"
CA41746	Murphy, J. R.	"Diphtheria Toxin-Related Peptide Hormone Gene Fusions"
CA40987	Rohrschneider, L. R.	"Tumor Cell Biology of the fms Oncogene Proteins"
CA40394	Verma, I. M.	"Use of Oncogene Related Products for Therapy"
AIDS		
CA43447	Bolognesi, D. P.	"Clinical Studies on Prevention and Intervention in AIDS"
LIPOSOMES		
Preclinical		
CA24553	Huang, L.	"Targeting of Liposome to Tumor Cells"
CA37528	Hwang, K. L.	"Controlled Release of Liposomal Contents in Macrophages"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
LIPOSOMES - Preclinical (Continued)		
CA38043	Lachman, L. B.	"Biological Studies of Human Interleukin-1"
CA39448	Matthay, K. K.	"Treatment of Bone Marrow by Antibody-Directed Liposomes"
CA42065	Regen, S. L.	"New Synthetic Carriers for Antitumor Drugs"
CA35340	Papahadjopoulos, P. D.	"Liposome-Mediated Intracellular Delivery In Vitro"
CA40149	Schroit, A. J.	"Liposome Activated Macrophages for Therapy of Metastasis"
CA42992	Kleinerman, E. S.	"Liposome Therapy--A Potential Adjuvant for Childhood Osteosarcoma"
TUMOR VACCINES		
Preclinical		
CA29592	Kahan, B. D.	"Active Specific Immunotherapy in Man"
Clinical		
CA39248	Berd, D.	"Augmentation of Human Immunity by Cyclophosphamide"
CA40352	Livingston, P. O.	"Immunization of Melanoma Patients with Gangliosides"
CA36233	Mitchell, M. S.	"Specific Active Immunotherapy of Human Melanoma"
LYMPHOKINES OTHER THAN IL-2		
Interferons - Preclinical		
CA44262	Esteban, M.	"Mechanisms of Action of Interferon"
CA26475	Fleischmann, W. R.	"Modulation of the Anti-Tumor Effect of Interferon"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
LYMPHOKINES OTHER THAN IL-2		
Interferons - Preclinical (Continued)		
CA39039	Friedman, R. M.	"A Mechanism of Action of Interferon"
CA38661	Rubin, B. R.	"Interferons-Properties, Action and Patient Prescreening"
CA39478	Lvovsky, E. A.	"Cancer Treatment by Interferon Inducer and Radiation"
CA43201	Ozer, H.	"Immunoregulatory Effects of the Interferons"
Interferons - Clinical		
CA43212	Foon, K. A.	"Mechanisms of IFN Action in Hairy Cell Leukemia"
CA26966	Meyers, J. D.	"Human Leukocyte Interferon After Marrow Transplant"
Interleukin-1 - Preclinical		
CA38043	Lachman, L. B.	"Biologic Studies of Human Interleukin-1"
CA45143	Gaffney, E. V.	"Malignant Cell Growth Modulation by Interleukin-1"
CA46259	Gallicchio, V. S.	"Control of Hematopoietic Toxicity With Interleukin-1"
Lymphotoxin - Preclinical		
CA39093	Granger, G. A.	"Lymphocyte Released Cell-Toxin"
Tumor Necrosis Factor - Preclinical		
CA44365	Sehgal, P. B.	"Tumor Necrosis Factor Induces a New Regulatory Cytokine"
B-Cell Cytokines - Preclinical		
CA42006	Choi, Y. S.	"Human B-Cell Growth Factor"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
LYMPHOKINES OTHER THAN IL-2		
B-Cell Cytokines - Preclinical (Continued)		
CA45148	Maizel, A. L.	"Biomolecular Nature of Human B-cell Growth Factor"
CA35845	Sidman, C. L.	"Purification and Immunobiology of B-cell Maturation Factors"
Other Lymphokines - Preclinical		
CA39441	Jones, C. M.	"Studies of a Human Monocyte Cytotoxicity Factor"
CA41063	Remold, H. G.	"Development of Cell Lines for Production of MIF and MAF"
CA40567	Schnaper, H.W.	"Inhibition of Tumor Cell Growth by the Lymphokine SIRS"
LAK +/- IL-2		
Preclinical		
CA42962	Mazumder, A.	"Immunotherapy of Cancer By Lymphokine Activated Cells"
CA39489	Pauly, J. L.	"Interleukin-2 Mediated Regression of Breast Tumor"
CA47097	Ochoa, A. C.	"Long-term LAK Cells: Effectors and *In Vivo* Relevance"
Clinical		
CA39489	Mier, J. W.	"In Vivo Effects of Recombinant Human Interleukin"
CA43950	Mier, J. W.	"Immunobiology of LAK Cells"
CA43977	Stenzel, K. H.	"Renal Cell Carcinoma: Adoptive Immunotherapy"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
BCG		
Preclinical		
CA15325	Gray, G. R.	"Anti-Tumor Active Components of BCG Cell Walls"
Clinical		
CA42327	Lamm, D. L.	"BCG Immunotherapy in Bladder Cancer"
AUTOLOGOUS SPECIFIC ANTI-TUMOR CYTOXIC CELLS		
Preclinical		
CA30558	Cheever, M. A.	"Specific Immunotherapy of Murine Tumors"
CA39286	Mastrangelo, M. J.	"Developing T-cell Clones Cytoxic to Sarcomas"
CA32155	Reinisch, C. L.	"Functional Clones in Fatal Lymphoproliferative Disease"
CA37113	Wolmark, N.	"Murine Studies in Surgical Adjuvant Adoptive Immunotherapy"
CA49231	Shu, S.	"Adoptive T-cell Immunotherapy of Nonimmunogenic Tumors"
Clinical		
CA43244	Kradin, R. L.	"Clinical Use of T-Lymphocytes for Therapy of Lung Cancer"
TUMOR ANTIGENS		
Preclinical		
CA33049	Oettgen, H. F.	"Monoclonal Antibodies in the Treatment of Cancer"
CA42508	Reisfeld, R. A.	"Molecular Profile of Melanoma and Neuroblastoma Antigens"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
INVESTIGATIONS OF MATURATION OR DIFFERENTIATION INDUCERS		
Preclinical		
CA35999	Chiao, J. W.	"Maturation Induction of Human Leukemic Cells"
CA42006	Choi, Y. S.	"Human B-cell Growth Factors"
CA17393	Donahoe, P. K.	"Mullerian Inhibiting Substance"
CA38627	Sonenberg, M.	"Growth Hormone and Differentiation"
CA47589	Santoli, D.	"Growth Factor-Dependent Normal and Leukemic Cell Growth"
BONE MARROW TRANSPLANTATION		
Preclinical		
CA43026	Stewart, F. M.	"Alternative Sources of Hematopoietic Renewal"
Clinical		
CA23766	O'Reilly, R. J.	"Marrow Transplantation in Leukemia and Blood Diseases"
CA18221	Storb, R. F.	"Marrow Grafting for Treatment of Hematologic Malignancies"
CA36725	Vallera, D. A.	"Immunotoxins in Human Bone Marrow Transplantation"
METASTASIS		
Preclinical		
CA30726	Niederkorn, J. Y.	"Immunologic Modulation of Ocular Tumor Metastasis"
CA38768	Pantazis, C. G.	"Epigenetic Mechanisms in Metastasis Modulation"

TABLE I (CONTINUED)

GRANTS LISTED BY TOPIC AREA

TOPIC GRANT NUMBER	PRINCIPAL INVESTIGATOR	GRANT TITLE
TOXINS		
Preclinical		
CA24628	Nowotny, A. H.	"Relation of Structure to Function in Endotoxins"
CA41746	Murphy, J. R.	"Diptheria Toxin Related Peptide Hormone Gene Fusions"
CA42354	Maxwell, I. H.	"Tumor Cell Killing by Expression of a Toxin Gene"
SBIR		
Phase I		
CA47153	Brown, R. L.	"Serum-free Medium for LAK, NK Cells and Monocytes"
Phase II		
CA39870	Huston, J. S.	"Engineering of Antibody Binding Sites to Tumor Antigens"
CA39942	Mix, T. W.	"A New Large Scale Affinity Separation Technique"
CA44246	Sobol, R. E.	"Monoclonal Antibody Anti-Idiotype Tumor Vaccines"
OTHER TOPICS		
Preclinical		
CA47324	Cox, D. C.	"Murine Tumor Immunotherapy Using Non-infectious Reovirus"

TABLE II

BIOLOGICAL RESPONSE MODIFIERS PROGRAM
BIOLOGICAL RESOURCES BRANCH
CONTRACTS PROJECTED FOR FUNDING IN FY 88

CONTRACTS	Estimates ($K)
Preclinical	
Chemical Coupling of Cytotoxic Agents to Tumor Reactive Monoclonal Antibodies	$ 439
Characterization and Analysis of Proteinaceous Materials	120
Collection, Storage, Distribution, and Quality Assurance of BRM	428
Animal Lymphokine Evaluation	133
Preclinical Assessment of Monoclonal Antibodies	260
Large Scale Production of MoAb	587
Harlan/Sprague-Dawley	287
Clinical	
Master Agreement Orders	1,270
Phase I Contracts	1,600
Total	$2,254

TABLE III

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

INSTITUTIONS CURRENTLY HOLDING BRMP CLINICAL TRIALS MASTER CONTRACTS

Cleveland Clinic Foundation
Dartmouth-Hitchcock Medical Center
Duke University Medical Center
Fred Hutchinson Cancer Research Center
Georgetown University
George Washington University
Hahnemann University
Illinois Cancer Council
Memorial Sloan-Kettering Institute for Cancer Research
Mount Sinai School of Medicine
New England Medical Center Hospitals, Inc.
New York Medical Center
Ohio State University Research Foundation
Oregon Health Sciences University
Thomas Jefferson University Medical College
University of Alabama in Birmingham
University of California, Los Angeles
University of California, San Diego
University of Colorado
University of Maryland Cancer Center
University of Michigan Medical Center
University of North Carolina, Chapel Hill
University of Pittsburgh
University of Southern California
University of Virginia
University of Wisconsin Clinical Cancer Center
Vanderbilt University
Virginia Commonwealth University
Yale University School of Medicine

TABLE IV

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

PHASE I CLINICAL TRIALS FUNDED BY MASTER AGREEMENT ORDERS

Institution	Agent
Georgetown University	Leukocyte Interferon*
Sidney Farber Institute	Leukocyte Interferon*
Northern California Cancer Program	Leukocyte Interferon*
University of California, Los Angeles	Lymphoblastoid Interferon*
Duke University	Lymphoblastoid Interferon*
University of Wisconsin	Lymphoblastoid Interferon
University of California, San Diego	Thymosin*
Fred Hutchinson Cancer Research Center	Thymosin*
George Washington University	Thymosin
Sloan-Kettering Institute	Thymosin*
Northern California Cancer Program	Thymosin*
Vanderbilt University	MVE-2*
Ohio State University	MVE-2*
University of California, Los Angeles	Anti-T Cell Monoclonal Antibody*
University of California, San Diego	Anti-T Cell Monoclonal Antibody*
University of Southern California	Anti-T Cell Monoclonal Antibody*
Fox Chase Cancer Center (Jefferson Medical College)	Anti-T Suppressor Cell Antibody*
Pittsburgh Cancer Center	Anti-lymphoma Monoclonal Antibody and IL-2
Ohio State University	rInterferon-beta and gamma*
University of California, Los Angeles	rInterferon-beta and gamma*
University of Southern California	rInterferon-beta*
University of Wisconsin	rInterferon-gamma*
Duke University	rInterferon-gamma*
University of Wisconsin	rIFN-beta and rIFN-gamma - 2 trials
Yale University (University of Pittsburgh)	rIFN-alpha and rIFN-gamma
Illinois Cancer Council	Anti-T Cell Monoclonal Antibody Radio-Labeled Conjugate
University of California, San Diego	Anti-T Cell Monoclonal Antibody Conjugate*
Fred Hutchinson Cancer Research Center	Anti-melanoma Monoclonal Antibody and IL-2
Memorial Sloan Kettering Cancer Center	$F(ab')_2$ Fragments of Anti-melanoma Monoclonal Antibody
Memorial Sloan Kettering Cancer Center	Anti-melanoma Monoclonal Antibody and IL-2
Cleveland Clinic	Anti-melanoma Monoclonal Antibody and r-IFN-a*
Cleveland Clinic	Anti-melanoma Monoclonal Antibody and DTIC

TABLE IV (Continued)

BIOLOGICAL RESPONSE MODIFIERS PROGRAM

BIOLOGICAL RESOURCES BRANCH

PHASE I CLINICAL TRIALS FUNDED BY MASTER AGREEMENT ORDERS

Institution	Agent
University of Wisconsin	Anti-ovarian Monoclonal Antibody and rIFN-gamma
University of Southern California	Anti-lymphoma Monoclonal Antibody and rIL-2
University of Alabama at Birmingham	Anti-melanoma Monoclonal Antibody and rIL-2
University of Texas M.D. Anderson Can. Ctr.	Anti-melanoma Monoclonal Antibody and IFN-alpha
Mt. Sinai Cancer Center	Anti-lymphoma Monoclonal Antibody and rIL-2
University of Wisconsin	IL-2 (Recombinant) - 2 trials*
Illinois Cancer Council	IL-2 (Recombinant) - 2 trials
University of Wisconsin	LAK cells and Cyclophosphamide
	LAK cells and Cyclophosphamide and IL-2
Ohio State University	IL-2 and LAK Cells
University of Wisconsin	rIL-2 and LAK Cells
Cleveland Clinic	rIL-2 and TIL
University of California, Los Angeles	rIL-2 and Thoracic Duct Lymphocytes
Pittsburgh Cancer Center	rIL-2 and LAK Cells
Thomas Jefferson University	rIL-2 and Cytotoxic T-Cells
Cleveland Clinic	GM-CSF-Activation of Macrophage
University of California, Los Angeles	GM-CSF-Therapeutic Effects of IV vs Sub Q
New York University	GM-CSF-Macrophage Activation

PHASE II CLINICAL TRIALS FUNDED BY MASTER AGREEMENT ORDERS

Institution	Agent
Georgetown University	Lymphoblastoid Interferon*
University of California, Los Angeles	Lymphoblastoid Interferon*
Duke University	Lymphoblastoid Interferon*
Sloan-Kettering Institute	Lymphoblastoid Interferon*

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* Completed trials

SUMMARY REPORT

LABORATORY OF MOLECULAR IMMUNOREGULATION

October 1, 1987 to September 30, 1988

INTRODUCTION

Headed by Dr. Joost J. Oppenheim, it is the mission of the Laboratory of Molecular Immunoregulation (LMI) to investigate at the cellular, biochemical, and molecular level, the intercellular and intracellular processes that regulate host defense mechanisms, including: interaction of cytokines with specific cell surface receptors; the membrane, cytoplasmic, and nuclear events which occur after interaction of these molecules with their receptors; the lymphokine/ cytokine modulation of cellular functions that participate in host defense; the effects of Biological Response Modifiers (BRMs) on immunoregulatory pathways and the development of new BRMs that enhance host defense mechanisms; and the role of immunoregulatory pathways in the pathophysiology of diseases. the LMI officially consists of two sections: Lymphokine and Immunobiology. However, operationally the LMI actually consists of four research groups that are engaged in distinct, but thematically related research activities. The main research activities of each of the LMI groups during the past fiscal year will be summarized.

RESEARCH MANAGED THROUGH THE OFFICE OF THE CHIEF

Three of the LMI investigators, Kouji Matsushima, M.D., Ph.D., Joost J. Oppenheim, M.D. and Scott K. Durum, Ph.D., are engaged predominantly in research studies concerning the contribution of inflammatory cytokines to host defense.

Dr. Matsushima is at present a Visiting Scientist who heads a group consisting of two postdoctoral fellows and two guest researchers that are engaged in investigating the mechanism of production of interleukin 1 (IL 1) at the molecular and biochemical level. This group is studying the interactions of IL 1 with cell membrane receptors, identifying factors such as glucocorticoids and prostaglandins that regulate expression of IL 1 receptors, and defining the properties of this receptor. Dr. Matsushima and his coworkers are also studying a number of intriguing post-receptor intracellular effects of IL 1 and related cytokines such as tumor necrosis factor (TNF) and interferon β2 (IFN β2 or IL 6). These include effects of cytokines on the ornithine decarboxylase pathway, the induction by cytokines of manganese superoxide dismutase, and the induction of an as yet unidentified pp65 protein and other intracellular molecules.

Dr. Matsushima has pursued collaborative studies with Dr. K. Onozaki of the Tsukuba Medical School in Japan concerning the mechanism of the in vitro cytostatic effects of IL 1 on some tumor cell lines. These studies have led to observations that interactions of cytokines such as IL 1 and TNF or IL 1 and IFN β can have a profound synergistic effect on terminal differentiation of some hematopoietic tumor lines.

Dr. Matsushima has also collaborated with Drs. T. Yoshimura and Edward Leonard of the Laboratory of Immunobiology, NCI. This relationship has enabled

Dr. Matsushima to purify and partially sequence a novel cytokine, designated monocyte-derived neutrophil chemotactic factor (MDNCF). Dr. Matsushima has cloned the cDNA for MDNCF and collaborated with Dr. Ettore Appella of NIAID, NIH to have active MDNCF synthesized in vitro. MDNCF has also been expressed by mammalian cells in Dr. Matsushima's laboratory and by E. coli in collaboration with Dr. M. Yamada of the Dainippon Pharmaceutical Corporation. Dr. Matsushima has established that IL 1 and TNF induce cells to produce MDNCF. A patent application has been submitted for this new inflammatory cytokine.

Dr. Oppenheim, in collaboration with Dr. Ruth Neta, Section of Experimental Hematology of the Armed Forces Radiation Research Institute, observed that pretreatment with IL 1 protects mice from lethal doses of irradiation. This collaborative study of the in vivo effects of these cytokines has been extended to include an evaluation of the effects of a number of different cytokines, the effect of therapy with cytokines following irradiation, the beneficial effects of combining cytokine treatment with bone marrow grafts and the ameliorative effect of cytokines on toxic doses of chemotherapy. A number of other investigators, including Dr. Dan L. Longo, Dr. Ron Gress of the Immunology Branch, NCI, and Dr. Jack Pearson of the LEI, have collaborated on various aspects of this study.

Dr. Durum is an independent senior investigator who currently supervises the research of three postdoctoral fellows and works with one guest researcher. He is interested in gaining a better understanding of the immune response. Consequently, he has been studying the in vitro effect of cytokines such as IL 1 on the activities of T lymphocytes. He has therefore investigated the effect of IL 1 on the growth and differentiation of T cell clones. He has identified a few clones that are dependent on IL 1 for growth and many clones that actually have the capacity to produce IL 1. Dr. Durum has established an auto-stimulating role for IL 1 in some T cell clones and cell lines by using inhibitors of IL 1. Unexpectedly, his studies have led him to find that IL 1 actually also has stimulating effects on murine antigen presenting cells (APC). IL 1 induces APC to express a non-Ia cell surface molecule which is essential for their T cell activating functions. Furthermore, T cells produce an as yet unidentified factor which promotes IL 1 production by APC. This IL 1-inducing factor is a novel lymphokine. Consequently, Dr. Durum has hypothesized that the effects of IL 1 in T cell activation are bi-directional. In an attempt to determine whether T cells produce IL 1 in vivo, Dr. Durum has succeeded (using in situ hybridization techniques in normal and immunized mice) in showing that non-lymphoid cells in various lymphoid tissues express mRNA for IL 1. Finally, Dr. Durum has been engaged in a very provocative collaborative study with Dr. Mark Smith of the LBP to evaluate the effect on cells of intracellular injections of various cytokines. Although they did not succeed in showing that IL 1 had any intracellular activity, the investigators were able to demonstrate that intracellular administration of human IFN gamma does lead to the expression of Ia antigens on the surface of the injected murine cells. However, Ia expression also develops on noninjected cells distant from the injected cells. This is not due to leakage of IFN gamma into the extracellular milieu since human IFN gamma is not active on mouse cells. Consequently, the investigators have postulated the induction of another mediator and have shown that GM-CSF is a likely candidate.

IMMUNOBIOLOGY SECTION

The Immunobiology Section: conducts studies on the intracellular events responsible for the differentiation of lymphocytic and myelocytic cells and their activation to exert antitumor effector functions; studies the structure-function relationship between RNA secondary structures, transport stability and gene expression; conducts studies on the changes in cell surface and intracellular markers during the process of cell differentiation, activation, and lymphokine receptor interaction; investigates the role of cytokines, such as IFN, colony stimulating factor (CSF), and IL 1 and of oncogenes, such as fos, ras, myc and raf, in cell growth and differentiation; and analyzes the role of DNA structure in the control of lymphokine gene expression.

Luigi Varesio, Ph.D., the Acting Head of the Immunobiology Section has been focusing his efforts on the molecular basis for macrophage growth and differentiation and the acquisition of antitumor activity. In order to facilitate these studies, Dr. Varesio, together with his three postdoctoral fellows and two guest researchers, has established immortalized murine macrophage cell lines by transfecting normal bone marrow cells with recombinant retroviruses expressing both the oncogenes v-myc and v-raf but not with the individual v-onc molecules. This has provided Dr. Varesio with homogeneous populations of functional macrophages for studies of intracellular events following macrophage activation by various ligands. Dr. Varesio has shown that the functional response of the macrophage lines to cytokines was intact. Moreover, he has observed that the expression of the transforming viral genome could be inhibited by picolinic acid and IFN gamma by decreasing the stability of the viral mRNA. These results indicate that post-transcriptional events may be important in controlling viral gene expression in macrophages.

Dr. Varesio has investigated the molecular events associated with the expression of cytotoxicity by macrophages. He has established that the pathways of macrophage activation by IFN α/β or IFN gamma are different, since the former require active protein synthesis and protein kinase C activity to induce cytotoxic macrophages, whereas IFN gamma is effective in the presence of protein kinase C and protein synthesis inhibitors. However, studies of the RNA metabolism in cytotoxic macrophages revealed that the same alterations of ribosomal RNA metabolism were induced by IFN α/β and by IFN gamma suggesting the existence of common molecular targets of different activation pathways. IFN α/β or gamma all cause a down-regulation of synthesis of 28S ribosomal RNA, but have no effect on 18S rRNA. Since the secondary structure of rRNA precursors contains stable double stranded structures, it was postulated that rRNA precursors could activate IFN-induced double stranded rRNA dependent enzymes. Dr. Varesio established in cell free systems that rRNA is endowed with this activity. These results indicate that non-viral RNA can activate double stranded RNA dependent enzymes and provides a functional connection between IFN-induced increases in rRNA precursors, increases in double stranded RNA dependent enzymes and expression of macrophage function. These data indicate that the activation of macrophages to a cytotoxic stage is associated with the changes in rRNA metabolism and indicate that, in macrophages, the complex enzymatic interactions that coordinate the expression of the ribosomal genes is an intracellular target of IFN action. Consequently, Dr. Varesio provides expertise and leadership in studies of the contribution of macrophages and cytokines that regulate their functions in host defense.

Howard A. Young, Ph.D. is the resident molecular biologist of the LMI and Senior Investigator in the Immunobiology Section. Dr. Young supervises several postdoctoral fellows and works with one guest researcher. He has been investigating the regulation of IFN gamma gene expression by manipulating the structure of human genomic IFN gamma DNA and introducing these altered DNA constructs into mouse T lymphoblastoid cells, fibroblasts or B cells. This approach has enabled Dr. Young to establish that each distinct cell type analyzed displays different control mechanisms for the regulation of expression of the exogenously introduced interferon gene. These control mechanisms include both positive and negative regulation of mRNA transcription as well as post-transcriptional regulation of cytoplasmic mRNA expression. Thus, analysis of interferon gamma genomic DNA structure with respect to identification of regions involved in the control of gene expression provides the opportunity to understand different methods of gene regulation in a single gene system.

Dr. Young, in collaboration with Dr. John Ortaldo of the LEI, has also established that large granular lymphocytes (LGL) produce IFN gamma in response to a single stimulus such as IL 2 or PMA. Since these cells are also important components of LAK cell cultures, experiments have been carried out to differentiate these cells from T cells utilizing molecular technology. Dr. Young has found that LGL only produce a truncated, non-functional 1.0 kb mRNA of the T cell receptor β chain gene. Further studies have determined that CD3-LGL also fail to produce functional T cell receptor α and gamma chain mRNA transcripts. These data clearly indicate that these LGL do not utilize the T cell receptor gene products in target cell recognition and killing.

In order to determine a mechanism for potential differences in the utilization of the T cell receptor genes by LGL and T cells, the T cell receptor β chain DNA structure in LGL, T cells, B cells, and monocytes were compared. Dr. Young has analyzed at the DNA level the methylation status of the T cell receptor β chain gene in order to determine if genomic DNA modification of a lymphocyte specific gene can be utilized as a distinct marker to differentiate these cell populations. Utilizing the restriction enzymes, Msp 1 and Hpa 2, he has observed that the T cell receptor β chain gene in T cells is substantially hypomethylated while the gene in LGL is significantly more methylated, with the DNA pattern indicating that CD3- LGL may represent a diverse, mixed population of cells. Furthermore, he has found that the gene in B cells and monocytes is almost completely methylated but homogeneous in contrast to the pattern seen in LGL. These data indicate that methylation of specific DNA sequences correlates with active gene transcription and rearrangement and may be an important controlling factor in both of these processes involving the T cell receptor β chain gene. Thus, Dr. Young concludes that the results of his studies on LGL readily distinguish these cells from T lymphocytes.

LYMPHOKINE SECTION

The Lymphokine Section investigates the mechanism of action at the biochemical and molecular level; the therapeutic usefulness of lymphokines and other lymphocyte-derived growth and differentiation factors in the treatment of cancer; monitors the effects of treatment with lymphokines on immune responses; and investigates the role of oncogenes and proto-oncogenes in the modulation and alteration of immune system function and development.

William L. Farrar, Ph.D., supervises three postdoctoral fellows and works with two guest researchers. One of the two main themes of Dr. Farrar's studies is to gain a better understanding of the signal transduction pathways induced by lymphokines such as IL 2, CSF and IL 3 in lymphoid and myeloid cells. These include the activation of two distinct kinase systems and identification of a 68 kDa phosphorylated substrate; inhibition of adenylate cyclase activity; participation of GTP-binding proteins; synthesis of a phosphorylated diadenylate which facilitates DNA polymerase α activities; and phosphorylation of a 40S ribosome S6 protein which regulates the rate of protein elongation. In addition, Dr. Farrar is characterizing the lymphokine induced sequence of gene expression involved in the control of the cell cycle. The DNA binding proteins are to be identified, and the means by which binding proteins that act on enhancer elements regulate gene transcription are to be elucidated. In order to pursue these problems, Dr. Farrar has been utilizing antisense oligonucleotides to specifically inhibit the proliferative effects of lymphokines.

Dr. Farrar has also been interested in the interactions between the nervous and immune systems. He has confirmed studies showing that human T lymphocytes as well as neural tissues express the mRNA for pro-opiomelanocortin (POMC) in response to mitogenic stimuli. In addition, T cells and LGL also have opiate receptors and show responses to the β-endorphin ligand. On the other hand, rat brain cells express receptors for IL 1 as well as the T4 binding site for HIV. In addition, rat brain cells express mRNA for IL 1β, and some localized neurons in discrete areas of the brain also expressed the mRNA for IL 3. Thus, there appears to be a reciprocal network of shared receptors and ligands in the immune and nervous systems. However, the extent of the network and its physiological role remain to be established.

Francis W. Ruscetti, Ph.D., Head of the Lymphokine Section, supervises a group consisting of two postdoctoral fellows and one guest researcher. Dr. Ruscetti's research focuses on the effect of cytokines such as transforming growth factor β (TGF β), IL 2, IL 3 and other CSF on the growth and differentiation of normal and neoplastic hematopoietic cells. Dr. Ruscetti has studied the receptor interactions and post receptor events, including oncogene expression, induced by some of these cytokines. Of particular interest are his studies showing that TGF β is a negative regulator of hematopoietic cell growth, and that TGF β1 and TGF β2 are equipotent inhibitors. More importantly, TGF β had a selective effect on hematopoiesis by inhibiting the growth of only early human and murine progenitors. TGF β acted directly on the cells to block multipotent cell development but not unipotent cell growth. IL 3 stimulation of the cells stimulated increased expression of TGF β receptors on the cell surface and increased the secretion of latent TGF β protein. Thus, the ability of TGF β to block hematopoietic progenitor cell growth depends on the differentiated state of the cell. Consequently, TGF β appears to be a regulator of early hematopoiesis. Also, fresh and cultured lymphoid leukemic cells were insensitive to TGF β inhibition, whereas normal lymphoid cells are acutely sensitive to TGF β. Thus, this loss of negative regulation may play a role in the growth of some leukemic cells. The possible therapeutic effects of TGF β on leukemic cells are being evaluated.

Dr. Ruscetti's other major research interest centers on the interactions between human retroviruses such as HTLV I, II and III (HIV-1) and leukocytes. Since these retroviruses are all associated with chronic, slowly progressive diseases, Dr. Ruscetti asked whether host-cell restriction of viral expression, resulting in little or no viral production, is involved in pathophysiology of these diseases. Dr. Ruscetti has therefore developed in vitro cell line models to study latency of both HTLV-1 and HIV-1. Examination of HTLV-1 infected B cells has shown the virus to be latent in these cells. Yet the integrated pro-virus is functional and can be activated to transform other T and B cells. In addition, the macrophage, which has been shown to be a latent reservoir of infection in AIDS patients, was examined for ability to restrict HIV-1 expression. THP-1, a macrophage cell line derived from a patient with monocytic leukemia, becomes naturally latent with time in vitro for HIV. Understanding of the mechanism of latency may provide a means of devising antiviral therapy based on inhibition.

A model of the pathogenesis of these viruses states that viral pathology is a result of the stimulation of inappropriate transcription of cellular genes. In the case of HTLV-1 oncogenesis, studies of the expression of several proto-oncogenes shows that only c-rel, whose oncogenic derivation, v-rel, causes lymphoid tumors in birds, is over-expressed in all HTLV-1 infected leukemic T-cells studied. The role of the transactivating gene product in this process is being studied. c-rel expression may be involved in HTLV-1 oncogenesis.

In addition to host cell effects on intracellular virus production, Dr. Ruscetti is studying the role of the host defense mechanisms in viral associated diseases. In collaboration with Dr. John Ortaldo, he recently demonstrated that LGL with natural killer activity are able to spontaneously lyse HTLV-1 and HIV-1 infected target cells. Moreover, IL 2 activated LGL demonstrate significantly enhanced activity against these viral infected T cells suggesting that cellular immunity could play a role in host defense against these viral-associated diseases. Thus, Dr. Ruscetti serves as the LMI and BRMP expert in studies of hematopoiesis, leukemia, human retroviruses and AIDS.

INTERRELATIONSHIPS BETWEEN LMI INVESTIGATORS

The research programs of each of the individual LMI investigators are thematically interrelated. Although each of the investigators is studying various aspects of the properties and activities of cytokines, there is little or no overlap in their specific areas of interest. For example, Dr. Matsushima's work focusses on the biochemical and molecular aspects of human cytokines such as IL 1, MDNCF and TNF, whereas Dr. Durum asks questions concerning the immunological role of these cytokines using in vitro and in vivo murine models. Dr. Varesio is primarily interested in molecular studies of monocyte/macrophages. Drs. Varesio and Young investigate the interaction of cytokines such as IFN gamma with cytotoxic macrophages and LGL respectively. In addition, Dr. Young is a ready collaborator who willingly acts as a molecular biology resource person for the other Biological Response Modifiers Program (BRMP) laboratories. Dr. Farrar provides other LMI investigators with expertise concerning cytokine induced signal transduction pathways, gene regulation and neuro-immune pathways. Finally, Dr. Ruscetti is the LMI expert on cytokines affecting normal and neoplastic hematopoietic cells and on the interaction of retroviruses such as HTLV 1 and HIV 1 with such cells.

Each of the LMI working groups holds weekly work seminars. All LMI investigators also meet on a weekly basis to review the results and plans of an ongoing study, report on scientific meetings or learn from an invited lecturer.

In addition, there are weekly BRMP-wide invited lecturers, staff meetings, yearly conferences and casual contacts at which LMI investigators have the opportunity to exchange information. As is evident from LMI publications, there is considerable collaboration of LMI investigators with one another, as well as with other BRMP, NCI, NIH, and non-NIH scientists. Inevitably there also is healthy competition between some LMI scientists, and they stimulate one another to venture into new areas and to tackle divergent problems. Nevertheless, the close proximity of LMI scientists fosters interchanges and provides a critical number of investigators interested in the role of cytokines in host defense against tumors.

LMI scientists also benefit from their close proximity and excellent interactions with the other cellular immunologists and molecular biologists in the BRMP. In addition, the monthly therapeutic application seminars held in conjunction with the clinical investigators of the Clinical Research Branch (CRB), BRMP provide additional impetus and fruitful interactions for the LMI scientists. This provides a forum which enables LMI scientists to contribute to formulating protocols for clinical evaluation of biologicals. There are also many mutually beneficial consultative contacts and interchanges between LMI scientist and the extramural scientist administrators in the Biological Resources Branch (BRB), BRMP. This facilitates our ability to obtain recombinant cytokines from biotechnology firms and fosters collaborative studies and information exchange with the extramural community. Finally, LMI scientists also participate in many meetings with non-BRMP scientists and have been responsible for organizing a number of international meetings on cytokines over the past few years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09289-03 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Restorative Role of Cytokines in Hematopoiesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. J. Oppenheim	Chief	LMI, NCI
Others:	K. Matsushima	Visiting Scientist	LMI, NCI
	A. Masuda	Visiting Fellow	OAD, NCI
	D. Longo	Associate Director	OAD, NCI
	R. Gress	Senior Investigator	NCI

COOPERATING UNITS *(if any)*

Armed Forces Radiobiology Research Institute, Bethesda, MD (R. Neta);
PRI, NCI-FCRF (I. Kedar, P. Tiberghien)

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously reported that prior administration of interleukin 1 (IL 1) or tumor necrosis factor (TNF) protects mice from lethal doses of irradiation. G-CSF, GM-CSF, IL 2 or interferon α,β (IFN α,β) prevent the lethal effect of >900 r dose of irradiation whereas IL 1 and TNF synergized as radioprotectors. Pretreatment with G-CSF or GM-CSF together with suboptimal doses of IL 1 also had synergistic radioprotective effects. The radioprotective effect of cytokines is associated with stimulation of bone marrow (BM) hematopoietic cells: 1) The recovery of nucleated BM cells and of hematopoietic progenitor cells were enhanced in IL 1 pretreated lethally irradiated mice. 2) In vivo treatment of normal mice with IL 1, for 24 hrs results in BM cell enlargement, increased cell cycling and increased capacity to proliferate in vitro in response to GM-CSF. We are currently showing positive effects of IL 1 treatment on isologous as well as allogeneic BM transplants. Isologous BM cells synergize with IL 1 to protect mice against supralethal doses (1500 r) of irradiation. IL 1 also enhances the survival of irradiated mice given low numbers of allogeneic BM cells. We are also evaluating the therapeutic effects of cytokine administration following midlethal doses of radiation. We have preliminary results suggesting that IL 1, IFN-gamma and TNF, but neither G-CSF nor GM-CSF significantly improve survival if given immediately after irradiation. Studies of the mechanism of radioprotection by IL 1 revealed that IL 1, in a variety of target cells, rapidly induces a p25 moiety which was purified to homogeneity and identified as mitochondrial manganese superoxide dismutase (MnSOD). However, IL 1 failed to induce other intracellular scavengers such as glutathione peroxidase and catalase. It is possible that MnSOD contributes to the radioprotective effects of IL 1.

PROJECT DESCRIPTION

PERSONNEL

Joost J. Oppenheim	Chief	OC, LMI, NCI
Kouji Matsushima	Visiting Scientist	OC, LMI, NCI
Akinori Masuda	Visiting Fellow	OC, OAD, NCI
Dan L. Longo	Associate Director	OAD, NCI
Ron Gress	Senior Investigator	NCI

MAJOR FINDINGS

The purposes of this study focus on the protective and therapeutic effects of cytokines in counteracting the deleterious effects of irradiation and chemotherapy in animal models. By protective effects we mean effects achieved by pretreatment with cytokines which somehow increases the tolerance of an animal to radiation or chemotherapy. On the other hand, by therapeutic effects of cytokines we mean effects obtained by treatment after radiation or chemotherapy which are presumably based on induction of restorative repair and recovery mechanisms.

Drs. Ruth Neta, AFRRI and Joost Oppenheim hypothesized that since LPS stimulates the cells of the RES to produce a multiplicity of cytokines, that immunomodulatory mediators such as interleukin 1 (IL 1), tumor necrosis factor (TNF), colony stimulating factors (CSF), interferons (IFN) may be responsible for this radioprotective effect of exogenous immunomodulators such as LPS. This hypothesis was tested and in a series of publications, we have reported that prior administration of IL 1 and TNF, but not G-CSF, IL 2 or IFN α,β have radioprotective effects. When administered together, IL 1 and TNF have additive or synergistic radioprotective effects suggesting that their mechanisms of action differ. This observation argues against IL 1 mediating the radioprotective effect of TNF, or the converse. The effect of combined treatment with IL 1 and TNF was also greater than that achieved with optimal radioprotective doses of LPS. This result suggests that the two cytokines can be administered at a more effective ratio, and that toxic effects of LPS itself can be circumvented by using the purified cytokines.

Although both GM-CSF and G-CSF did not protect against lethal doses of irradiation by themselves, they had synergistic radioprotective effects when administered in conjunction with suboptimal doses of IL 1. The synergistic interactions of TNF and IL 1 and CSF and IL 1 also suggest that these cytokines may act in concert on in vivo hematopoiesis. IL 1 does not have the ability to directly stimulate the subsequent growth of more differentiated hematopoietic cells and the possibility that its effects may be mediated by a cascade of other growth factors was investigated. Following the administration of radioprotective doses of 100 ng of IL 1 per mouse, elevated serum titers of CSF appear at 3 and 6 hrs after treatment. Assays for interferon in the same sera were negative. The results suggested that CSF but not IFN may be of importance in IL 1 mediated radioprotection. Like IL 1, TNF induces CSF and may thus indirectly promote hematopoiesis. The possibility that IL 1 and TNF may also protect non-hematopoietic tissues from the injurious effects of radiation needs to be evaluated.

IL 1 is more effective if given intraperitoneally or subcutaneously rather than intravenously. Single doses of IL 1 ranging from 100 - 500 ng IP protect C57Bl6 as well as a number of other mouse strains against 900 - 1200 r doses of radiation (DRF = 1.25). TNF is less potent than IL 1 and 5000 to 7500 ng doses of human TNF are needed to radioprotect mice (DRF = 1.15). However, recent data shows that higher dose of 10 μg IL 1β result in more radioprotection. The time of administration of IL 1 is critical, maximal effects are obtained by administering IL 1 20-24 hrs prior to irradiation, only minimal radioprotection is obtained if IL 1 is given 4 hrs prior to radiation and no radioprotection was obtained if IL 1 was given 48 hrs before or at the time of lethal radiation.

The necessity of a 20 hr time lag between the cytokine treatment and lethal irradiation for optimal radioprotection with IL 1 suggests that IL 1 has to condition the recipient to be radioprotective. The multiplicity of biological effects of IL 1 provides a number of possible mechanisms of radioprotection. IL 1 is a known inducer of prostaglandins which have radioprotective effects. However, radioprotection with IL 1 was not mediated by prostaglandin release, since treatment with either indomethacin or aspirin only inhibited the radioprotective effect of IL 1 to a limited degree.

The patterns of hematopoietic recovery in endotoxin treated animals closely resembles the hematopoietic recovery in animals receiving syngeneic bone marrow transplants. Consequently, the accelerated restoration of functional immunocytes and hematopoietic cells is believed to be a major factor in the survival of lethally irradiated mice. IL 1-treated, as compared to non-treated, mice five days after irradiation had greatly enhanced numbers of nucleated bone marrow cells and increased endogenous splenic colonies suggesting that IL 1 administration may stimulate bone marrow hematopoiesis. In fact, our studies of normal mice established that intraperitoneal administration of IL 1 within 24 hrs significantly increased the percentage of large cells in S+G+M phases of the cell cycle. In addition, the capacity of BM cells from IL 1 treated mice to proliferate in cell suspension cultures in response to GM-CSF was greatly increased. The observed induction of cell cycling with IL 1 correlates with the enhanced radioprotective effect of this cytokine.

THERAPEUTIC EFFECTS OF CYTOKINES

Our preliminary experiments show that IL 1 is also effective in promoting the hematopoietic recovery following mid-lethal doses of irradiation. A single injection of IL 1 after irradiation with an LD 95/30 dose promotes survival of up to 100% of C3H/HeN mice (Neta, et al.). The therapeutic effect was dose dependent and peaked with doses ranging from 1-5 μg IL 1. Thus much higher doses of IL 1 were needed for therapeutic than radioprotective effects suggesting that the preventive effects are mechanistically distinct from the reparative effects of IL 1. Although IFN-gamma had no radioprotective effect, when therapeutic doses of 1.25 μg of IFN-gamma were given IP after mid-lethal irradiation, we observed significantly improved survival. In contrast, doses of 5 μg TNF showed only limited therapeutic efficacy, while a broad dose range of G-CSF and GM-CSF, used within hrs after irradiation, were not effective in promoting survival following mid-lethal doses of irradiation.

The mechanisms by which these cytokines promote recovery from radiation remains unknown. We have performed one study designed to establish a role for repair enzymes. IL 1 stimulation of an IL 1 dependent lymphocyte cell line (D10G4.1) induced only modest increases in gene expression for DNA polymerase β which were proportional to increases in expression of genes for actin. The pathways of recovery from radiation damage differ from those operating in radioprotection since IFN-gamma was not active as a radioprotector in lethally irradiated mice. The fact that IL 1 is not as effective when used after irradiation as before irradiation in treatment of lethally or supralethally irradiated mice, given higher radiation doses, may indicate that a small number of surviving stem cells or progenitor cells may be required to serve as targets of IL 1.

In order to evaluate the radioprotective effect of IL 1 on patients receiving multiple doses of irradiation, mice were given IL 1 before, after and/or in between fractionated doses of radiation in the lethal range. Preliminary results were reassuring since all mice that received more than one dose of IL 1 survived.

Non-Hematopoietic Effects of Cytokines

The possibility that cytokines may protect cells and tissues in addition to hematopoietic tissues is being addressed by evaluating the therapeutic effects of isologous BM transplants given in conjunction with IL 1 to supralethally irradiated mice. Mice have been exposed to doses ranging from 1,250 to 2,000 r irradiation and treated with IL 1 along with isologous BM cells and the duration of survival determined. Mice are synergistically protected by BM cells and IL 1 against doses as high as 1,500 r but not 2,000 r suggesting GI toxicity may be diminished by IL 1. Experiments to establish the effects of giving IL 1 prior to as well as after BM transplantation are planned. These experiments should reveal whether IL 1 protects non-hematopoietic tissues and thus results in better survival of mice exposed to doses of irradiation that will cause gastrointestinal damage, if treated with BM cells and IL 1 in comparison with BM cells alone.

The Effect of Cytokines on Bone Marrow Allografts

The effect of cytokines on allogeneic BM transplants is being studied in the mouse model. C57B16 mice immediately after irradiation with 1,000-1,200 r have been given various doses of allogeneic (Balb/C) BM (T cell depleted) cells intravenously with or without a therapeutic dose (2-10 μg) of IL 1. Preliminary observations reveal that the IL 1 itself prolonged the survival of about 2/3 of the mice. From 2.5 to 10 x 10^6 allogeneic (T cell depleted) BM cells in a dose dependent manner prolonged survival of the recipient mice only for a transient period. Treatment of mice with IL 1 together with 5-10 x 10^6 allogeneic BM has led to prolonged survival of the majority of mice. Thus, therapy with IL 1 following irradiation improves the survival of 2/3 of the mice given suboptimal numbers of allogeneic BM cells.

The Effect of IL 1 on Lethal Chemotherapeutic Treatment

In collaboration with Drs. Jack Pearson of the LEI, BRMP have initiated a study of the ameliorative effect of IL 1 in cytoxan treated mice. Preliminary studies using survival as the end point revealed that administration of IL 1 prior to lethal doses of cytoxan accelerates the onset of lethal effects. The opposite effect was seen with WR2721 (a scavenger of oxygen intermediates) which by itself decreases the cycling of BM cells and transiently prolonged survival following lethal doses of cytoxan. Administration of IL 1 at a single 100 ng dose at 24 hrs or daily x 3 following a cytoxan dose prolongs survival transiently. In order to optimize the therapeutic effects such doses of IL 1 were therefore given in combination with WR2721 which was administered just prior to cytoxan. WR2721 given before cytoxan, together with IL 1 given after cytoxan, showed the most significant effects in promoting the survival of a fraction of the mice. However, these results still need to be optimized. The early death of the mice suggests that cytoxan is toxic to more tissues than the bone marrow. Experiments are in progress using higher doses of IL 1 that have proven therapeutic in irradiated mice.

Possible Radioprotective Intracellular Effects of Cytokines

A study concerning the fundamental mechanism of action of IL 1 that is being performed in collaboration with Drs. Akinori Masuda, Kouji Matsushima, and Dan Longo may have thrown some more light on the mechanism by which IL 1 may exert radioprotective effects. In order to identify the subcellular events induced by IL 1, the effect of IL 1 on protein synthesis by human A375 melanoma cells were examined. IL 1 selectively and predominantly induced a 25 kilodalton protein (p25) in A375 cells after 12 hr. Upon subcellular fractionation, p25 was exclusively located in the 10,000xg pelleted (mitochondria-enriched) fraction. To identify the p25 moiety, it was purified to homogeneity by sequential chromatography on DEAE-Sephacel and reverse-phase HPLC, and its amino acid sequence was determined. The amino-terminal sequence of the p25 moiety was identical to that of human manganese superoxide dismutase (MnSOD). The enzymatic activities of SOD were induced only in the mitochondria-enriched fraction of IL 1-treated A375 cells. IL 1 also induced MnSOD in normal human skin fibroblast and peripheral blood mononuclear cells whose growth was stimulated by IL 1. These results show that induction of MnSOD by IL 1 may be a common biological event in IL 1-responsive cells. On the other hand, IL 1 had no effect on the intracellular levels of glutathione peroxidase and catalase. The induction of a free-radical-scavenging mitochondrial SOD by IL 2 in normal cells perhaps contributes to the radioprotective effect of IL 1.

PROPOSED COURSE

The radioprotective effects of administering various recombinant cytokines prior to lethal irradiation is being evaluated. 1) The optimal time, dose and route of administration of the cytokines needs to be established. 2) Since in vivo pathophysiological responses generally result in the production of a number of cytokine activities, the protective and therapeutic effects of various combinations of cytokines should be evaluated. 3) Mice are used to obtain detailed data and miniature pigs (150-200 lbs) will be used to obtain information on larger animals in order to prepare for clinical studies in man. 4) In order to stimulate clinical therapy more closely, the effects of administering cytokines before, between and after multiple doses of radiotherapy must be explored. 5) Since higher doses of radiation and chemotherapy can be given in conjunction with the infusion of autologous bone marrow (BM) cells, the effect of administering cytokines together with isologous BM grafts in increasing the tolerance to higher doses of irradiation and drugs is to be examined. 6) Furthermore, the cytokines may have beneficial effects on engraftment of allogeneic BM cells. Eventually the effects of the protective and therapeutic cytokines on tumor bearing animals must be tested to determine whether they have deleterious tumor promoting activity.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09216-08 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Molecular Basis for Macrophage Activation and Immortalization

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Varesio	Visiting Scientist	LMI, NCI
Others:	M. Clayton	Microbiologist	LMI, NCI
	G. Cox	BTP Fellowship	LMI, NCI
	G. Gusella	Visiting Fellow	LMI, NCI
	I. Espinoza	Guest Researcher	LMI, NCI

COOPERATING UNITS (*if any*)

PRI, NCI-FCRF (D. Radzioch, M. Leyko, T. Musso); LEI, BRMP, DCT, NCI (R. Wiltrout); Instituto di Microbiologia Medica, Perugia, Italy (E. Blasi).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The use of recombinant retroviruses expressing activated oncogenes to immortalize/transform fresh hematopoietic cells has been investigated. It was established that: a) fresh murine bone marrow (BM) cells can be immortalized in vitro in the absence of exogenous growth factors; b) immortalization of BM cells requires the collaboration of two oncogenes (v-myc and v-raf) each of which is ineffective by itself; c) under these conditions only macrophages are immortalized; and d) the immortalized macrophage cell lines express the functional activities of normal peritoneal macrophages. Further analysis of the mechanisms by which transforming oncogenes affect the cellular response to environmental stimuli was conducted on NIH 3T3 fibroblasts transformed by a single oncogene. We found that H-ras can inhibit the induction of c-fos mRNA and protein in fibroblasts stimulated by phorbol esters. However, only H-ras and not K- or N-ras, dbl or trk oncogenes affected the responsiveness to phorbol esters of NIH 3T3. These results provided the first evidence of the existence of a biological difference among ras oncogenes and indicated that at least some of the effects of H-ras may be due to reduced c-fos expression.

The molecular events occurring during the activation of macrophages to a tumoricidal stage have been examined. We identified: a) stimulus-specific responses elicited by various activating agents which are evident during the early phases of macrophage activation, including augmentation of c-fos expression and of S-adenosylhomocysteine content; and b) common biochemical alterations expressed late during activation, namely a decrease in ribosomal RNA (rRNA) maturation and a consequent accumulation of rRNA precursors. We found that such rRNA precursors can activate double-stranded RNA-dependent enzymes via stable double-stranded secondary structures. We speculate that the interaction between these enzymes and the rRNA precursors plays a role in the activation and/or expression of tumoricidal activity by macrophages.

PROJECT DESCRIPTION

PERSONNEL

Luigi Varesio	Visiting Scientist	IS, LMI, NCI
Michael Clayton	Microbiologist	IS, LMI, NCI
George Cox	BTP Fellowship	IS, LMI, NCI
Gabriele Gusella	Visiting Fellow	IS, LMI, NCI
Igor Espinoza	Guest Researcher	IS, LMI, NCI

OBJECTIVES

The objectives of the research have been: 1) to characterize the biological and biochemical properties of macrophage cell lines immortalized by oncogenes; 2) to investigate the molecular events leading to the expression of cytotoxic activity by macrophages; 3) to examine the mechanisms controlling gene expression in non-proliferating, terminally differentiated cells; and 4) to determine whether activated oncogenes could affect the expression of other cellular genes.

MAJOR FINDINGS

I. Biological and Biochemical Properties of Macrophage Cell Lines Immortalized by v-myc/v-raf Oncogenes

We were successful in immortalizing macrophages from the bone marrow of various strains of mice by infection with recombinant retroviruses. The results of these studies can be summarized as follows:

1. Fresh murine bone marrow cells BM can be immortalized in vitro by retroviral infection in the absence of exogenous growth factors.

2. Immortalization of BM cells requires the collaboration between v-myc and v-raf oncogenes (carried by the recombinant retrovirus J2), each of which is individually ineffective.

3. J2-virus immortalizes macrophages selectively which can be grown in vitro (J2-Mϕ).

4. J2-Mϕ are indistinguishable from peritoneal macrophages on a functional basis. J2-Mϕ derived from genetically defective C3H/HeJ mice whose macrophages do not respond to endotoxins, retain endotoxin unresponsiveness, supporting the notion that J2 virus infection does not alter the functional activity of the cells.

5. J2 virus can transform macrophages from mouse fetal liver. However, the growth pattern of these macrophages differ from that of BM.

6. The cellular target of the J2 virus in BM and fetal liver is lost in culture within 12 hr. However, CSF-1 prolongs the survival of this cell in culture up to 48 hrs.

An interesting question raised by our results was why only bone marrow derived macrophages were immortalized by transfecting murine bone marrow with combinations of v-myc/v-raf. As a first attempt to address this issue, the integration site of the virus was studied. To our surprise, a restricted number of integration sites were detected suggesting that the site of integration may be important for the establishment of the cell lines.

The effects of cytokines on v-myc, v-raf-expressing macrophage cell lines was studied. We have established that the cells do not respond with proliferation to IL 2, although the cells are sensitive to IL 2, since IL 2 is a co-stimulus for the activation to cytotoxicity. We also assessed whether the cell lines were still susceptible to the growth inhibitory effects of cytokines such as IFN-γ. It was found that the IFN-γ can inhibit the growth of the macrophages suggesting that the viral protein does not affect growth inhibitory mechanisms. In addition, it was found that LPS is growth inhibitory at high doses and at lower concentrations can synergize with IFN-γ in blocking cell proliferation. Preliminary studies aimed at understanding the mechanisms responsible for the growth inhibition revealed a possible role of endogenously produced IL 1 and/or TNF in this process. The viral mRNA expression was not altered in IFN-treated macrophage cell lines. Inhibition of viral mRNA could be achieved only by exposure of the cells to IFN and other BRMs such as picolinic acid. These results indicated that under certain conditions viral expression could be inhibited, and preliminary evidence suggested that the decrease of viral mRNA could be accounted for by increased degradation of the transcript.

To pursue these investigations it became important to evaluate the v-myc and v-raf protein levels in the cells. Since anti-raf monoclonal antibodies were not available, such a reagent was prepared in collaboration with Dr. Ulf Rapp and Steve Giardina, utilizing as antigen a 30 Kd truncated raf protein produced in E. coli. The monoclonal antibodies produced is being characterized and it seems to recognize c- as well as v-raf by immunoprecipitation and Western blot analysis.

Experiments were performed to assess whether the macrophage cell lines were tumorigenic. In collaboration with Dr. R. Wiltrout we observed that the cell lines were tumorigenic in nude mice but not in conventional mice. The tumors growing in nude mice were due to proliferation of the infected cells rather than host cells transformed by the released virus.

II. Expression of c-fos in Fibroblasts Transformed by Oncogenes of the ras Family

The discovery of the need for a collaboration between v-myc and v-raf gene product for the immortalization of macrophages stimulated our interest in understanding the relationships among oncogenes and the mechanisms controlling their expression. Experiments were initiated to examine the influence of a transforming oncogene on the expression of a proto-oncogene, and for this purpose NIH 3T3 cells were initially used since they provided a simple and well characterized biological system for evaluating this issue. The c-fos expression was investigated for its proposed role in cell proliferation and differentiation of myeloid cells. The transforming oncogenes analyzed were H, K, N-ras as well as trk and dbl.

The initial results of these studies revealed the following: 1) Harvey-ras inhibited the induction of c-fos mRNA and protein by the PKC activator TPA; 2) N-or K-ras as well as the ras-unrelated oncogenes dbl and trk did not affect c-fos inducibility.

These results provided the first evidence that, despite the kinship of ras oncogenes, H-ras is biologically different from K- or N-ras and suggested that the mechanism by which H-ras induces transformation, may involve deregulation of c-fos expression.

III. Intracellular Events Leading to Activation of Tumoricidal Macrophages

We have analyzed 1) the early, stimulus-specific responses of macrophages to activating agents, as well as 2) the late, function-specific molecular changes related to the expression of tumoricidal activity.

A. Early Events

The biochemical pathways involved in the activation of macrophages by IFN differ depending upon the interferon type. IFN-γ can activate cytotoxic macrophages in the presence of cycloheximide, an inhibitor of protein synthesis. In contrast, IFN α/β requires active macrophage protein synthesis to induce cytotoxic activity. These findings indicated that IFN-γ can activate macrophages by interacting with preexisting substrates, whereas IFN α/β require the synthesis of new proteins to trigger the activation process. Attempts to identify the biochemical events initiated by IFN-γ and leading to macrophage activation revealed various changes in IFN-γ treated macrophages. An increase in the intracellular content of s-adenosylhomocysteine associated with activation of macrophages by IFN-γ has been demonstrated. Studies on c-fos expression in macrophages were performed since c-fos expression is an early response to stimulation and it has been implicated in macrophage differentiation. The levels of c-fos mRNA were dramatically increased by stimulation with phorbol myristate acetate (PMA), calcium ionophore, or 1-oleoyl-1-acetyl glycerol (OAG). Induction of c-fos mRNA by all the above agents followed similar kinetics, with a peak of mRNA 30 min after stimulation. These results demonstrated that c-fos mRNA can be augmented in fresh, terminally differentiated cells. Since the stimuli increasing c-fos mRNA are direct or indirect activators of protein kinase C, our data suggested that the Mφ c-fos mRNA is controlled by protein kinase C activation. PMA, calcium ionophore, and OAG were biologically active in Mφ. PMA and calcium ionophore induced respiratory burst and tumoricidal activity, respectively, whereas OAG and PMA were chemotactic for Mφ. These results indicate that c-fos mRNA augmentation is a stimulus-specific rather than a function-specific response connected to activation of protein kinase C. These results raised the question of the role for protein kinase C activity in macrophage activation. Experiments with inhibitors of protein kinase C indicated that activation of macrophages by IFN α/β requires active protein kinase C, whereas activation by IFN-γ was independent from protein kinase C. These observations are consistent with the finding that IFN-γ does not induce c-fos mRNA expression in macrophages. Moreover, these results provide additional evidence that the mechanisms by which IFNs induce cytotoxic macrophages differ depending upon the type of IFN.

B. Late Events

Despite the differences among the pathways triggered by distinct macrophage activators, we demonstrated that the decrease in RNA synthesis was a general characteristic of cytotoxic macrophages. A number of approaches were taken to investigate the nature of the decrease in RNA synthesis that we had previously found to be selectively associated with the expression of tumoricidal activity by macrophages. These studies led to the discovery that the ribosomal rRNA was specifically altered in macrophages expressing tumoricidal activity. In these cells, we identified an increase in the steady state levels of ribosomal RNA (rRNA) precursors leading to an unbalanced accumulation of mature rRNA. The accumulation of rRNA precursors was observed in macrophages activated in vivo as well as in vitro, irrespective of the activating agent used and we concluded that it was a metabolic event associated with and probably required for the expression of tumoricidal activity. The methylation of rRNA was also decreased in cytotoxic Mø suggesting a role for methylation in the maturation of rRNA precursors. These findings provided the first evidence for a post-transcriptional control of gene expression associated with macrophage activation. Moreover, they led to the identification of the accumulation of rRNA precursors as a biochemical step, shared by the different macrophage-activating pathways related to the expression of macrophage-mediated cytotoxic activity.

These results raised the question of the molecular connection between accumulation of rRNA and expression of tumoricidal activity by macrophages. To address this question, we investigated first the structure of the rRNA precursors. Computer analysis showed a complex secondary structure endowed with a very stable double stranded RNA structures. These findings let to the hypothesis that the double stranded RNA moieties of the rRNA precursors, could activate dsRNA-dependent enzymes which in turn may be involved in the expression of cytotoxic activity. The following approach has been initiated to verify this hypothesis. The first step was to demonstrate that the rRNA precursors can activate double stranded dependent enzymes. To obtain a pure preparation of rRNA precursors, rRNA genes were expressed in cell free transcription systems, the rRNA transcripts were purified and tested for their ability to activate a double stranded RNA-dependent enzyme the 2'5' oligoadenylate synthetase (2'5'A synthetase). Preliminary results suggest that some sequences of the ribosomal RNA precursors can activate 2'5'A synthetase and that this activity was due to the double stranded structure. These results provide clear evidence that intracellular RNA may activate double stranded RNA dependent enzymes. Moreover, they support the hypothesis of an active role played by the accumulation of rRNA in the expression of tumoricidal activity by macrophages.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09260-06 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Human Inflammatory Cytokines: Production, Properties, and Effects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Y. Kobayashi	Guest Researcher	LMI, NCI
	C. Larsen	Guest Researcher	LMI, NCI
	K. Hirose	Guest Researcher	LMI, NCI
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Laboratory of Molecular Immunoregulation

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Immunobiology Section

INSTITUTE AND LOCATION

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1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of IL 1 production have established that endotoxin-stimulated human monocytes rapidly synthesize, accumulate intracellularly and partly release IL 1 into culture media. Intracellular IL 1 is located mostly in the cytosol of monocytes and precursor IL 1α but not IL 1β is phosphorylated exclusively at serine residues. Phosphorylation promotes enzyme cleavage and the plasma-membrane binding capacity of precursor-IL 1α. We have studied post-receptor events and have determined that IL 1 rapidly phosphorylates 65 and 74 kDa cytosolic proteins at serine residues in glucocorticoid pretreated human PBMC. We have further investigated the basis for the growth inhibitory/cytocidal effect of IL 1 on several types of human tumor cell lines. Both in cells that are activated by IL 1 or that undergo cytocidal processes, IL 1 regulates a rate-limiting enzyme for polyamine synthesis, ornithine decarboxylase and induces a mitochondrial associated 25 kDa superoxide dismutase moiety.

We have purified novel neutrophil chemotactic factor (NCF) to homogeneity and determined the partial amino acid sequence. Based on the protein sequence, the cDNA was cloned and the complete nucleotide sequence for NCF was determined and amino acid sequence deduced. Both IL 1 and tumor necrosis factor both induce mRNA for NCF and NCF activity in human PBMC.

PROJECT DESCRIPTION

PERSONNEL

Kouji Matsushima	Visiting Scientist	IS, LMI, NCI
Joost J. Oppenheim	Chief	OC, LMI, NCI
Masahiro Shiroo	Visiting Fellow	IS, LMI, NCI
Ajoy Samanta	Guest Researcher	IS, LMI, NCI
Yoshiro Kobayashi	Guest Researcher	IS, LMI, NCI
Christian G. Larsen	Guest Researcher	IS, LMI, NCI
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Naofumi Mukaida	Guest Researcher	IS, LMI, NCI
Dan L. Longo	Associate Director	OAD, BRMP, NCI

OBJECTIVES

We are attempting to gain a better understanding of: 1) how intracellular cytosolic IL 1α and IL 1β are released and processed; 2) how signals are transduced by IL 1 receptors and result in altered gene expression; 3) how IL 1 inducible genes are regulated; 4) examine the cell biology of the effects of IL 1 on normal and malignant cells on which it acts; and 5) identify new cytokines involved in inflammation and tumor immunity.

MAJOR FINDINGS

1. Study of the Production of Human IL 1

a. Phosphorylation of intracellular precursor IL 1α and possible role of phosphorylation in the processing of pre-IL 1α.

The human IL 1 molecules (IL 1α and IL 1β) are post-transcriptionally cleaved from 31 kDa precursors to 18 kDa biologically active molecules. In the course of studies of post-translational modifications of human IL 1, we have observed using LPS stimulated human monocytes that the intracellular precursor IL 1α (pre IL 1α) to be phosphorylated at least 10 fold more than intracellular pre IL 1β. However, no ^{32}P-incorporation could be detected in the 18 kDa processed IL 1α and IL 1β. Analysis by thin layer chromatography revealed that the major phosphorylation site occurred at serine residues. The ^{32}P was incorporated into multiply cleaved precursors of IL 1α, which appeared in the absence of protease inhibitors. Since the smallest molecular weight pre IL 1α that was labeled with ^{32}P orthophosphate was 22 kDa, and the carboxyl terminal 18 kDa mature IL 1α molecules are resistant to protease cleavage, the phosphorylation site is presumably the serine residue located adjacent to a sequence of four basic amino acids located in the 4 kDa region at the amino terminus of the 22 kDa precursor IL 1α. This serine residue might also be a major phosphorylation site for a cAMP-dependent protein kinase.

This hypothesis was substantiated by the demonstration that a synthetic peptide analog of this region (residue 84 to 112) could be similarly phosphorylated in vitro by a cAMP-dependent protein kinase. Furthermore, a truncated pre-IL 1α (residue 64 to 271) and a fusion protein containing staphylococcal protein A and amino terminal half portion of pre IL 1α (residue 1 to 112), but not mature IL 1α (residue 113 to 271), could also be phosphorylated in vitro by a cAMP dependent protein kinase. There is no comparable amino acid sequence in precursor IL 1β which could be expected to be phosphorylated by a cAMP dependent protein kinase.

The physiological relevance of phosphorylation of pre IL 1α was investigated. Phosphorylation of recombinant truncated pre IL 1α greatly enhanced the susceptibility of this molecule to digestion by trypsin and promoted the conversion of pre IL 1α to the more biologically active IL 1. This finding suggests that the phosphorylation of serine residue close to the dibasic/tetrabasic amino acid sequence may facilitate the processing and/or release of IL 1α.

The effect of selective phosphorylation of pre IL 1α on the possible association of pre IL 1α with the plasma membranes was also studied. The binding of recombinant truncated pre IL 1α (residue 64 to 271) (which was phosphorylated in vitro by a cAMP dependent protein kinase) to phospholipids was studied. ^{32}P labeled-phosphorylated pre IL 1α binds to acidic phospholipids including phosphatidic acid (PA), phosphatidylserine (PS) and phosphatidyl inositol (PI), but not to other phospholipids. In contrast, unphosphorylated pre-IL 1α did not bind to PS. This binding required divalent cations; Ca++ or Mn++ but not Mg++, with half maximal binding to PS, at 0.3 mM of Ca++. This binding was reversible, because EGTA could elute phosphorylated pre-IL 1α from PS, EGTA or alkaline phosphatase could also elute IL 1 activity from LPS-stimulated human monocytes without loss of viability. Based on Western blotting analysis and neutralization assays, the released IL 1 molecules consisted solely of 31 kDa pre-IL 1α. We also observed that EGTA has the capacity to elute ^{32}P labeled phosphorylated pre-IL 1α from viable monocytes. Taken together, these observations suggest that phosphorylated pre-IL 1α binds to the outer surface of the plasma membranes of monocytes in a Ca++ and phospholipid dependent manner.

b. Release and processing of IL 1α and β

In order to learn how cytosolic IL 1 molecules are released and processed, a pulse-chase experiment was performed using LPS-stimulated human monocytes. When freshly isolated human monocytes were labeled with ^{35}S-methionine for 2 hr in the presence of 10 μg/ml LPS and chased for 1 to 4 hr, the intracellular IL 1α and β molecules consisted predominantly of pre-IL 1 (31 kDa) whereas equal amounts of released pre-IL 1s and mature IL 1s (18 kDa) were present in the culture media. An intermediate form of IL 1α (24 kDa) was also detected in the media. No further processing of these soluble pre- and intermediate-IL 1s into mature IL 1 occurred in culture media during the chasing period. When monocytes stimulated with LPS for 24 hr were used for a similar pulse-chase experiment, again no significant processing was observed for either pre-IL 1α or IL 1β in the culture media. These data indicate that processing is not coupled with release of IL 1 molecules and that the processing, therefore, occurs during or just after the release of IL 1 molecules from the cells. In addition, agents which have been reported to block the release of secretory proteins, such as chloroquine, ammonium chloride, and monensin, did not block the release of IL 1 molecules from LPS-stimulated human monocytes. These data suggest that the mechanism by which IL 1 molecules are released from LPS-stimulated monocytes is distinct from the usual secretory process.

2. Intracellular events following IL 1 receptor signal transduction

We previously reported glucocorticoids upregulate IL 1 receptor expression on human PBMC and fibroblasts. In order to establish the functionality of glucocorticoid-induced IL 1 receptor on PBMC and elucidate the early biochemical events following IL 1 receptor interaction, we studied the effect of IL 1 on protein phosphorylation in normal human PBMC. Carrier-free recombinant human IL 1α (as little as 1 ng/ml) specifically and predominantly induced phosphorylation of an acidic (pI = 5 to 5.5) 65 kDa protein (pp65). The induction of phosphorylation of pp65 by IL 1 was enhanced by treating PBMC with prednisolone (10^{-7}M to 10^{-5}M) for 5 hr, which correlated well with the increase the number of IL 1 receptors by glucocorticoids on PBMC. The phosphorylation occurred within 1 min and reached a maximum at 10 min. Thin layer chromatography of hydrolysate of ^{32}P-labeled 65 kDa protein purified from IL 1 stimulated PBMC cytosol showed that only serine residues were phosphorylated. Fractionation of IL 1-stimulated PBMC homogenates by ultracentrifugation showed pp65 to be located in the cytosol, suggesting that pp65 is not the IL 1 receptor itself. Both unphosphoryalted and phosphorylated forms of 65 kDa protein have been purified to homogeneity by sequential chromatography including Sephacryl S-200 gel filtration, HPLC anion exchange, and HPLC hydroxyapatite from the cytosol of IL 1 stimulated prednisolone treated PBMC. Amino acid sequence analysis of a CNBr cleaved peptide from pp65 showed that pp65 has a novel protein sequence. cDNA cloning is in progress.

We have recently identified the phosphorylation of serine residues of several other cytosolic minor proteins (such as 50 kDa, 74 kDa, and 92 kDa) in PBMC after IL 1 stimulation. Moreover, we have identified a cytosolic protein kinase whose activity is significantly augmented following IL 1 stimulation by using unphosphorylated 65 kDa protein as an in vitro substrate. This kinase is distinct from protein kinase C, cAMP-dependent protein kinase, or calmodulin kinase. The purification of these phosphoproteins and a novel protein kinase is in progress.

3. Antitumor effects of IL 1

We have studied the intracellular molecular events after treatment of tumor cells with IL 1. ODC activity was markedly suppressed by IL 1 in those cells on which IL 1 exerts an antiproliferative effect, such as a human melanoma cell line (A375) and human mammary tumor cell lines (MCF-7 and T-47D). On the other hand, ODC activity was stimulated in cells that are stimulated to proliferate in response to IL 1, such as a mouse helper T lymphocyte cell line (D10.G4.1), a human natural killer cell line (YT) and a human glioblastoma cell line (U373

MG). The effect of IL 2 on ODC activity anteceded and correlated with its effect of DNA synthesis. Furthermore, putrescine, a product of the ODC reaction and a precursor of polyamines, was able to overcome most but not all the antiproliferative action of IL 1 in A375 melanoma cells, which were the most sensitive to suppression by IL 1.

However, putrescine did not reverse the cytostatic effect of IL 1 on MCF-7 and T-47D cell lines. In contrast, putrescine, like IL 1, exhibited comitogenic activity on D10.G4.1 cells with concanavalin A. These data suggest that the regulation of ODC activity may be a key component in the antiproliferative and proliferative action of IL 1 in some types of cells. MnSOD was identified as a protein (p25) which is most dramatically induced by IL 1 in human melanoma cells. The cDNA for human Mn-SOD was cloned from the cDNA library of LPS-stimulated human monocytes constructed in lambda gt 10. Northern blotting analysis shows mRNA for MnSOD is induced within 1 hr after stimulating melanoma cells with IL 1. We did not observe the induction of catalase or glutathione peroxidase activities by IL 1 in these cells.

4. cDNA cloning for MDNCF

The amino acid sequence of MDNCF derived from LPS-stimulated PBMC conditioned media showed that MDNCF had an unique protein sequence. Therefore, cDNA cloning of MDNCF was performed using oligonucleotide probes. The cDNA library was constructed in the phage vector lambda gt 10 based on the mRNA from LPS-stimulated human monocytes. There is a 101 base 5′ untranslated region, a 297 base coding region, and long 1.2 kb 3′ untranslated region. The initial AUG condon begins an open reading frame that encodes a 99 amino acid polypeptide. The amino terminal sequence is highly hydrophobic for a typical signal peptide. The mature form of the protein starts with serine at residue 28. The deduced amino acid sequence of MDNCF contains no N-glycosylation site. The deduced amino acid sequence shows striking similarity to several platelet derived factors, a v-src induced protein (9E3), a melanoma growth factor (gro), and a γ-interferon inducible protein (γ-IP 10).

The availability of the MDNCF cDNA probe enabled us to identify inducers of MDNCF mRNA expression in human PBMC. MDNCF mRNA was increased more than 10 times with 1 hr after stimulation with LPS, IL 1, or TNF. There was no induction of mRNA for MDNCF by γ-interferon α-interferon, or interleukin 2. Furthermore, we also determined that LPS, IL 1, and TNF stimulated PBMC to produce biologically active MDNCF.

SIGNIFICANCE

The study of the production of IL 1 suggests that the mechanism of IL 1 release/secretion and plasma membrane association are probably unique. Our data suggest that phosphorylation of pre-IL 1α may increase the sensitivity of pre-IL 1α to the processing enzyme(s) and phosphorylated pre-IL 1α binds to plasma membranes in Ca++ dependent manner. Identification of intracellular phosphoproteins whose phosphorylation is selectively augmented by IL 1 stimulation has established that IL 1 receptor is associated with a serine kinase. Purified unphosphorylated form of 65 kDa protein can be used to identify the protein kinase(s) which is responsible for phosphorylating 65 kDa protein in the cells.

Although the biological significance of the induction of MnSOD by IL 1 has not yet been established, the induction of MnSOD may reflect a secondary response to superoxide, hydrogen peroxide, and/or hydroxyl radical generation in melanoma cells generated in the course of induced cytocidal effects of IL 1 on these tumor cells. The radioprotective activity of IL 1 to normal cells may also be partly due to induction of this enzyme by IL 1. Identification, purification, cDNA cloning and of a novel neutrophil chemotactic factor has established that this molecule is clearly distinct from IL 1 or TNF, but that this molecule can be induced by IL 1 and TNF. Recombinant NCF will facilitate the work on the receptor for NCF and establish its biological effects in vivo.

PROPOSED COURSE

It is the overall aim of this laboratory to investigate the mechanism by which human inflammatory cytokines such as interleukin 1 (IL 1) contribute to host defense and tumor immunity. We are therefore studying the mechanism of production, intracellular molecular events, and properties of closely related cytokines.

1. Study of the production of human IL 1. It is the purpose of this project to learn how intracellular cytosolic IL 1α and β molecules are released and processed. Identification of processing site(s), processing enzymes, and mechanism of membrane binding of IL 1 molecules will be pursued.

2. Study of signal transduction mechanisms mediated by IL 1 receptor and post-receptor molecular events.

a. Identification of cytokines and other agents which up- and down-regulate the expression of IL 1 receptors and their mechanism of action.

b. Identification, purification, and cDNA cloning of cytosolic and nuclear proteins which are phosphorylated after IL 1 stimulation and identification of protein kinases which mediate the protein phosphorylation.

c. Study of the regulation of the genes [e.g. monocyte derived neutrophil chemotactic factor (MDNCF) and manganese-superoxide dismutase (MnSOD)] induced by IL 1. Genomic cloning of these genes, identification of regulatory regions, and binding of nuclear proteins to these regions will be studied. The direct and indirect effects of the above IL 1 regulated-phosphoproteins and protein kinases on the expression of these genes will be also examined.

3. Identification, purification, and cDNA cloning of novel monokines and cytokines.

a. Study of the production of MDNCF by fibroblasts, keratinocytes and other types of cells in vitro and in vivo.

b. Characterization of the receptor for MDNCF and the receptor mediated signal transduction mechanism of this ligand.

c. Study of biological activities of MDNCF in vitro and in vivo.

d. Expression and large scale production of recombinant MDNCF by E coli and mammalian cells.

e. Chemical synthesis of MDNCF polypeptide and determination of smallest active fragment of MDNCF.

f. Purification and cDNA cloning of other chemotactic factors, such as monocyte-specific chemotactic factor and T4 positive T lymphocyte-specific chemotactic factor.

4. In vitro and in vivo effects of IL 1, chemotactic factors, and other cytokines on tumor cell growth.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09283-04 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Expression in Immune Effector Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. A. Young	Expert	LMI, NCI
Others:	V. Ciccarone	IRTA Fellow	LMI, NCI
	K. Mace	IRTA Fellow	LMI, NCI

COOPERATING UNITS *(if any)*

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3.0	3.0	0.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is divided into two parts: 1) control of specific lymphokine (i.e. interferon γ) gene expression; and 2) analysis of T cell receptor DNA structure during the growth and differentiation of immune effector cells (i.e. T cells, LGL, B cells and monocytes).

1. We have identified specific regions of human interferon γ genomic DNA which can significantly effect the expression of this gene by transfecting DNA constructs containing IFN-γ genomic DNA linked to chloramphenicol acetyltransferase into a variety of murine cell lines. At least two regions of the DNA which contain enhancer elements have been identified. In addition, we have observed that the DNA sequences in the first intron of the gene are required for mRNA expression in a murine B cell line and may be involved in cellular responses to calcium mobilization.

2. Comparison of the methylation pattern of the T cell receptor beta chain gene (T β) in different lymphoid populations has revealed additional distinct differences between these cell populations. Hypomethylation of the gene was found to correlate with gene expression and to be highest in T cells and lowest in B cells and monocytes. Specific differences in methylation patterns were noted when T cell DNA was compared to LGL DNA and B cell DNA was compared to monocyte DNA. In addition, the T cell receptor β chain gene in immature mouse thymocyte (dlyl) DNA was found to be more highly methylated than the DNA isolated from CD4+ or CD8+ thymocytes.

PROJECT DESCRIPTION

PERSONNEL

Howard A. Young	Expert	IS, LMI, NCI
Valentina Ciccarone	IRTA Fellow	IS, LMI, NCI
Kenneth Mace	IRTA Fellow	IS, LMI, NCI

MAJOR FINDINGS

1. Control of Human Interferon-γ Gene Expression.

We have chosen interferon-γ (IFN-γ) gene expression as a model system for analysis of the control of gene expression in T cells and large granular lymphocytes (LGL). We have found that T cells and LGL are the only two cell types to produce this protein and that IFN-γ mRNA transcription occurs after stimulation of these cells with agents such as interleukin 2 (IL 2) or lectins (e.g. PHA). Thus, the goals of this project are to define, at the molecular level, the regions of human interferon gamma genomic DNA which are involved in the transcriptional activation and mRNA stability of this gene. More specifically, we wish to define those regions of the non-coding portion of the genome which are required for transcriptional activation of the gene by IL 2, phorbol myristate acetate (PMA) and by triggering of specific T cell surface markers, such as CD3 and CD28.

The human IFN-γ genomic DNA was isolated as part of an 8.6 kb BamH1 DNA fragment. This DNA contains approximately 2.3 kb of DNA 5' to the coding region and approximately 1.3 kb of DNA 3' to the coding portion of the gene. Upon introduction of this gene into a murine T lymphoblastoid cell line, we observed an increase in cytoplasmic IFN mRNA after IL 2 or PMA treatment which takes place in the presence of cycloheximide, indicating that protein synthesis is not required for induction by either agent. PMA but not IL 2 treatment also resulted in a stabilization of the IFN mRNA, as half-life determination in the presence of actinomycin D indicates that the IFN mRNA has a half-life of 2-3 hrs after no treatment or IL 2 treatment and a half-life of 4-6 hrs after PMA treatment. Expression of the transfected gene is inhibited by cyclosporin, dexamethasone or forskolin, similar to that observed for the endogenous gene. Thus, the overall biological activity of the human IFN-γ genomic DNA indicates that most if not all of the regions of DNA required for regulated expression of the gene are contained in the 8.6 kb BamH1 DNA fragment. Further structural analysis of the gene has indicated that enhancer activity can be detected in the 5' non-translated genomic DNA as well as the internal 2.3 kb Xba I fragment.

The stable introduction of the parental IFN genomic DNA into mouse L cells or NIH 3T3 cells produced an unexpected result. Transfected fibroblasts did not spontaneously produce human IFN-γ nor was production detected after PMA treatment. However, treatment of the cells with 1 ug/ml cycloheximide for 4-7 hrs followed by replacement of the cycloheximide with fresh medium for 10-18 hrs resulted in the appearance of human IFN in the culture supernatant. Nucleic acid analysis of these cells has indicated that there is a high level of spontaneous transcription occurring in the absence of any inducing agents and that the RNA could only be detected in the nuclear fraction and was larger that the final processed mRNA. Cycloheximide treatment resulted in the appearance in the cytoplasm of processed mRNA which was subsequently translated into biologically active protein. PMA treatment during the cycloheximide treatment did not increase protein or cytoplasmic RNA expression and IFN-γ expression was not dependent upon the growth stage of the cell. IFN-γ expression in these cells was blocked by cyclosporin or dexamethasone. Analysis of IFN-γ genomic DNA deletions has indicated that the presence of the AUAA sequences in the 3' untranslated region of the parental RNA is not the cause of the absence of processed cytoplasmic mRNA. Stable transfection of IFN-CAT constructs into the mouse fibroblasts has indicated that strong enhancer elements exist in the 5' non-coding region and 2.3 kb internal Xba I DNA fragment. This observation is consistent with the strong spontaneous expression of the parental gene in this cell type and current studies are designed to specifically identify the nucleotide sequence of these enhancer elements.

The current studies presented here concerning the regulation of IFN-γ gene expression offer an important opportunity to dissect the role of a specific immunoregulatory molecule in immune system development. By fully identifying the regulatory regions that exist in the genomic DNA and elucidating how these regions are involved in the induction as well as repression of gene expression, one can better understand the pattern of gene expression in vivo and predict how to manipulate the in vivo expression of this gene. With the approaches described here, these studies will permit a molecular and biological understanding of the regulation and role of IFN-γ gene expression in development and immune system function.

2. Analysis of T Cell Receptor DNA Structure During the Growth and Differentiation of Immune Effector Cells.

The purpose of this research project is to develop molecular markers for the characterization and differentiation of human large granular lymphocytes (LGL) from other immune effector cell populations. We have analyzed at the DNA level the methylation pattern of the T cell receptor beta chain gene in freshly isolated LGL, T cells and other lymphocyte populations to determine if the methylation pattern of this gene can serve as a marker for specific cell populations, provide information concerning the lineage of these cell populations and suggest possible mechanisms for controlling the functional expression of this gene during lymphocyte development and maturation.

The methylation pattern of the T cell receptor beta chain constant region genes has been analyzed in DNA isolated from LGL, T cells, B cells and monocytes. Based on the published nucleic acid sequence, we have determined that there are 31 Msp I (Hpa II) sites in this region of DNA with most of these sites being clustered in the J1 and J2 regions of the genomic DNA. DNA from different lymphocyte cell types was first analyzed with the restriction enzymes Msp I and

Hpa II which recognize the sequence CCGG. Msp I cleaves the DNA regardless of the methylation state of the internal C while Hpa II only cleaves the DNA if the internal C is not methylated. Thus, the patterns of restriction enzyme fragment size after cleavage with these two enzymes provides information as to the state of DNA methylation of this genomic DNA region. The results of cleavage of DNA with these enzymes and hybridization with a cDNA probe homologous to both the C beta 1 and C beta 2 regions has indicated that there is a clear difference in the methylation pattern when the different lymphocyte DNAs are compared. B cell and monocyte DNA are not cut by Hpa II indicating that this region of DNA in those cells is heavily methylated. In contrast, T cell DNA is readily cleaved by this enzyme, indicating that numerous areas of this DNA are not methylated. LGL DNA appears to contain an intermediate pattern of methylation indicating that some of the regions which are hypomethylated in T cell DNA are methylated in LGL DNA but that other regions of DNA which are methylated in B cell and monocyte DNA are not methylated in LGL DNA. Furthermore, since the sum of the individual DNA bands in the LGL DNA is greater than that of the T cell DNA, this data suggests that LGL are a heterogenous population with regard to T cell receptor beta chain gene methylation. These results further suggest that LGL are more related to T cells than to B cells or monocytes and perhaps methylation of the T cell receptor beta chain C region may be a useful marker to differentiate these populations.

More detailed analysis of the methylation site differences between LGL and T cells was carried out using multiple restriction enzyme digestions. The DNA was first cut with restriction enzymes which cut the genomic DNA into known fragments ranging anywhere from <1 kb to 12-15 kb. The DNA was then cut with Msp I or Hpa II to determine whether or not the CCGG sites within these DNA fragments were methylated. Distinct differences in the hybridization patterns of LGL and T cell DNA were observed after digestion with two restriction enzymes. These results confirmed the earlier observations that LGL appear to have more methylated sites in the J1 region and the C2 region of their DNA than is seen in T cells. However, both LGL and T cells as well as B cells and monocytes appear to have little methylation in their HTF island (high CG rich region) near the J2 region of their DNA. These results indicate that methylation of specific regions of the T cell receptor beta chain gene is distinct in LGL DNA as compared to T cell DNA and can be a useful marker for classifying malignancies of unknown lymphoid origin. Furthermore, it also suggests that methylation of hypomethylation of this DNA region may play a role in recombination events which result in the generation of a functional T cell receptor beta chain mRNA. Since LGL do not transcribe a functional beta chain mRNA or rearrange their beta chain genes, methylation of specific sites within this region of genomic DNA may inhibit the recombination processes from taking place.

The approaches described above have provided useful methods for identifying specific differences between LGL and T cells and examining the biochemical and molecular events which occur upon external stimulation of these cells. It is anticipated that the continued analysis of specific gene structure for the purpose of understanding how gene expression is controlled will provide important information regarding the function and maturation of distinct immune effector cell populations.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09287-04 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytokines and the Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. K. Durum	Senior Staff Fellow	LMI, NCI
Other:	L. Takacs	Visiting Fellow	LMI, NCI
	K. Muegge	Guest Researcher	LMI, NCI
	F. Aiello	Guest Researcher	LMI, NCI
	L. Bristol	IRTA Fellow	LMI, NCI

COOPERATING UNITS (if any)

PRI, NCI-FCRF (M. Smith); LBP, BRMP, DCT, NCI-FCRF (H-F. Kung); LMI, BRMP, DCT, NCI-FCRF (H. Young); DCBD, NCI (J. Berzofsky, R. Hodes).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Most peptide hormones react with receptors on target cells and are then internalized. We have examined the possibility that there is a function for internalized cytokines. Various cytokines were microinjected into appropriate target cells, and single cell assays were then performed to analyze the response. We observe that microinjection of interferon-gamma (IFN-gamma) into macrophages induces synthesis of granulocyte-macrophage colony-stimulating factor, and this subsequently induces Ia-expression on other macrophages. Microinjection of tumor necrosis factor (TNF) killed several types of cells. On the other hand microinjection of interleukins 1 and 2 did not elicit detectable responses from appropriate target cells. Based on the novel observations with IFN-gamma and TNF, we propose a new role for some cytokine receptors: transport of the cytokine to an intracellular site of action.

Interleukin 1 (IL1) has been previously implicated in the activation of T lymphocytes, and has been thought to be provided by antigen-presenting cells (APC) to the T cell, along with processed antigen and Ia. We previously observed that the opposite was true for T cell clones: they produce their own IL1. We now observe that this IL1 derived from T cells performs a crucial, previously unrecognized role in antigen presentation: IL1 induces the appearance of essential structures on antigen-presenting cells. Thus, if APC are fixed with a cross-linking agent prior to exposure to IL1, they fail to stimulate T cell proliferation; IL1 treatment results in expression of new structures, permitting fixation of the APC.

PROJECT DESCRIPTION

PERSONNEL

Scott K. Durum	Senior Staff Fellow	IS, LMI, NCI
Laszlo Takacs	Visiting Fellow	IS, LMI, NCI
Kathrin Muegge	Guest Researcher	IS, LMI, NCI
Francesca Aiello	Guest Researcher	IS, LMI, NCI
Lynn Bristol	IRTA Fellow	IS, LMI, NCI

OBJECTIVES

1) We have asked whether internalization of peptide hormones has physiological significance.

2) We have analyzed the role of IL1 in T cell activation from a new perspective, the effect of IL1 on APC.

METHODS EMPLOYED

1) Recombinant cytokines were directly introduced into the cytoplasm of various target cells by microinjection using a microcapillary tube. For microinjection, cells were attached to glass coverslips using concanavalin A (for lymphoid cells) or allowed to attach naturally (macrophages and fibroblasts). IL1 was injected into a T cell line, IL2 into a different T cell line, IFN-gamma into macrophages, and TNF into macrophages and fibroblasts. Responses of individual microinjected cells were monitored using autoradiographic methods to detect Ia expression and DNA synthesis.

2) APC were treated with various recombinant lymphokines. APC were then fixed with the cross-linking agent ECDI, and used to stimulate proliferation or lymphokine secretion from various T cell clones.

MAJOR FINDINGS

1) We have shown that IFN-gamma, microinjected into macrophages, induced GM-CSF synthesis, and this in turn induced Ia on other macrophages. TNF, microinjected into fibroblasts or macrophages, was toxic. IL1 and IL2 did not have detectable activities when microinjected into target cells.

2) We observe that fixation of APC with ECDI results in APC that can produce lymphokines, but cannot proliferate. Prior activation of APC induce essential new structures, such that APC could be fixed, yet stimulate T cell proliferation. We have coined the term "IMAS" for "inducible membrane accessory structures". Different types of APC required different stimuli to express IMAS

(macrophages required IL1 or LPS, B cells required IL4). IMAS is probably a membrane structure, distinct from known cytokines or Ia, and is synthesized de novo.

SIGNIFICANCE

1) Previously, peptide hormones were thought to act on target cells by binding surface receptors which then transduced biochemical signals resulting in changes in interior regulatory molecules (e.g. cAMP or Pi). Our demonstration that IFN-gamma and TNF have activities inside target cells has introduced a new biological concept: some cell surface receptors for peptide ligands must have a role as transporters, rather than just as transducers, as previously thought.

Several facets of the biology of individual cytokines have also been elucidated by these studies. Human IFN-gamma does not act on murine macrophages when exposed to the outside of the cell because it is not recognized by the receptors on the surface; however, it has an intracellular action, implying a second recognition system. We also made the surprising observation that microinjected TNF kills macrophages, even though this is the cell type that synthesizes TNF. It is known that TNF is synthesized as a pro-peptide (76 amino acids longer than the mature peptide), that is processed to the mature form after externalization. We suggest that the pro-piece functions to mask the toxic effect of TNF during synthesis.

2) Our studies reveal a new facet to the process of T cell activation by APC. An APC in "ground state" is capable of stimulating a partial response, lymphokine production, from a T cell, but the T cell does not receive sufficient stimulation to proliferate. The lymphokines then "activate" the APC, resulting in rapid expression of new structures, IMAS, on the APC membrane. IMAS is required to induce proliferation of the T cell. The potential clinical significance of these findings is that antibodies to IMAS may induce immunological tolerance, permitting the transplantation of foreign tissues.

PROPOSED COURSE

1) To pursue the observations that some cytokines have intracellular actions in the target cells, we will examine the next step in their action within the cell. Thus, IFN-gamma, acting inside a macrophage, induces GM-CSF production. We will first evaluate the simplest hypothesis: perhaps IFN-gamma interacts directly with the regulatory elements of the GM-CSF gene. If it does not interact with the gene directly, we will look for internal receptors. For TNF, the next step in the intracellular mechanism of TNF killing of cells could be related to the digestion of DNA, previously noted by others. Therefore, we will examine whether TNF interacts intracellularly with a DNase, resulting in activation of this enzyme. If this fails, we will also look for internal receptors for TNF. Other growth factors and peptide hormones, besides IFN-gamma and TNF will be studied for intracellular effects. Also, instead of microinjecting the peptides, as we have previously done, we will inject antibodies against them,

then expose the cell to the peptide given from the outside. The latter type of experiment is important because, if the antibody blocks, this will indicate that internalization of the ligand is necessary. Based on our findings, for some peptides, like IFN-gamma and TNF, surface receptors seem to function primarily for transport. But perhaps for others, like IL1 and IL2, membrane receptors have two functions: transduction plus transport. This could be revealed by injecting antibodies to IL1 and IL2, and would explain why, in our previous experiments, microinjecting IL1 or IL2 did not deliver a sufficient signal.

2) Our primary goal is the identification of murine IMAS. Several candidates for IMAS include the murine counterparts to the human cellular adhesion molecules, ICAM-1 and LFA-3. We will test several potential antibodies to these structures, available from collaborators, and will also use cDNA probes to determine whether IMAS corresponds to these structures. If IMAS appears to be unique, we will attempt to produce our own antiserum in rabbits, then use this antiserum to study the protein(s) and identify the cDNA in an expression system.

3) Several major goals of the laboratory involve shifts in the level of study from the cellular to the molecular level. Most of the lab personnel are now employing some molecular techniques to answer questions. In the next year, we will undertake the cDNA cloning of several molecules we have identified: IMAS (discussed above), a novel lymphokine that induces IL1 production, and unique mRNAs induced by IL1 in T cells.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09251-06 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Interactions of Human Retroviruses with Hematopoietic and Adherent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. W. Ruscetti	Senior Investigator	LMI, NCI
Others:	G. K. Sing	Visiting Fellow	LMI, NCI
	N. C. Lohrey	Microbiologist	LMI, NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

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SECTION

Lymphokine Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
1.75	.75	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acquired immunodeficiency syndrome (AIDS) is associated with a viral (HIV-I) mediated progressive depletion of the helper/inducer T4+ T cell subset, whereas acute T cell leukemia (ATL) is associated with viral (HTLV-I) mediated growth of the same T cell subset. To better understand the host cell effects on viral replication, we have developed in vitro cell line models to study latency of both HTLV-I and HIV. Examination of HTLV-I infected B cells from ATL patients has shown the virus to be latent in these cells. No viral mRNAs are being transcribed yet the integrated provirus is functional and can be activated to transform other T and B cells. When the virus infects T-cells, the virus is expressed; when it infects B cells, it is not expressed. In addition, the macrophage, know to be infected in AIDS patients was examined for ability to restrict HIV-I expression. THP-1, a macrophage cell line derived from a patient with monocytic leukemia, was permissive for HIV-I expression. However, these infected cells become naturally latent for HIV 4-6 weeks after infection. HIV-I and HTLV-I are among a class of viruses that have a unique mechanism of stimulating viral transcription. Both viruses make proteins which act in trans to positively regulate viral transcription. Studies of the transactivation mechanism in both of these latently infected cultures showed that transactivation of viral transcription is markedly inhibited. This indicates that the host factors play a role in this transcription and these cell either are deficient in positive regulators or possess negative regulators of viral transcription.

PROJECT DESCRIPTION

PERSONNEL

Francis W. Ruscetti	Senior Investigator	LS, LMI, NCI
Garwin K. Sing	Visiting Fellow	LS, LMI, NCI
Nancy C. Lohrey	Microbiologist	LS, LMI, NCI

MAJOR FINDINGS

I. Models for Restricted Viral Expression or Latency for HTLV-I and HIV-I.

The human T-cell leukemia viruses, HTLV-I and HTLV-II, contain a genetic region termed x, whose protein products function to regulate virus gene expression. The larger x region protein, p40, activates viral transcription in trans (trans-activation) directed by the viral long-terminal repeat (LTR). HTLV-I has recently been shown to naturally infect B as well as T-lymphocytes. The replicative and trans-activating properties of one such virus (HS) was examined. The HTLV-I infected B-cell line developed from a patient with HTLV-I associated adult T-cell leukemia demonstrated a lack of trans-activation, replication, absence of viral RNA and proteins including p40. Transformation of fresh cord blood and tonsillar T and B cells by co-cultivation with the lethally irradiated HS cell line showed that viral the genome was not defective. Studies on the HS virus using individual T and B cell lines from the same donors showed that in T-cell lines normal trans-activation occurred but that in B-cell lines, although they could be used to transform other cells, neither trans-activation, viral RNA nor viral proteins were present. Transient co-transfection assays using an exogenous HTLV p40 expression vector plus the HTLV LTR-directed CAT plasmid revealed a depressed transactivation in B-cell lines containing the HS viral genome compared to other T and B cell lines. The block to transcriptional activation of the virus was removed after treatment the cells with PMA or gamma irradiation. Thus, some B cells appear to be non-permissive for HTLV-I viral replication. These results suggest that HTLV-I gene expression can be regulated by the cellular environment.

Similarly, a human monocytoid leukemic cell line, THP-1 which possesses several normal functions including (1) phagocytosis, (2) accessory cell function, and (3) cytokine release was use to develop a model for HIV-I latency. A mycoplasma-free THP-1 culture was studied for cytological and immunological effects of HIV-I infection. A prototype HIV-I virus isolated from PBL from an AIDS patient and passaged into HUT-78, a T-cell line, was used to infect THP-1. After two weeks, THP-1 culture media was positive for RT and p24 antigen and the cells became T4 negative and p24 positive. HIV-I infected THP-1 cells were as positive for phagocytosis of yeast particles, accessory cell activation of T-cell proliferation and interleukin 1 release as the uninfected culture. In addition, the differentiative effects of PMA on THP-1 cells were not affected by HIV infection, nor was the viability of THP-1 cells. THP-1 cells released only a third of the virus release from the HUT-78. However, HIV produced in THP-1 cells had typical biological effects. As few as 100 THP-1 cells co-cultured with 20,000 cells of MT-2 or CEM (T-cell lines) resulted in complete cell death in five days. Six to eight weeks after the infection of THP-1, the culture become RT and p24 negative and T4 antigen was found on the cell surface. HIV can be induced in these non-producing THP-1 cell cultures suggesting that HIV has become latent in these cultures. These results are similar to reports of HIV-I infection of normal monocytes and suggest that monocyte/macrophage system is an important reservoir of persistent HIV infection in man.

II. Expression of c-Rel in Normal and Neoplastic Human Lymphoid Cells

The transforming gene v-rel, of the leukemogenic reticuloendotheliosis virus, is derived from avian cellular DNA and is unrelated to any other known oncogene human c-rel transcripts are restricted to hematopoietic cells and tissues, particularly lymphoid cells. Surprisingly, they are more abundant in more mature differentiated cell types. In collaboration with Elise Brownell and Nancy Rice, we have studied c-rel expression in human cells. A 530 base pair segment of the human c-rel gene, containing the human homologs of exxon 4 and 5 of the turkey gene was used to study patterns of expression in human leukemic cell lines and normal lymphocyte populations. Unlike most other proto-oncogenes, c-rel is expressed most abundantly in differentiated lymphoid cell types. c-rel expression was seen in both human T and B cells with resting B cells showing must greater levels than in resting T-cells of either T4+ helper or T+8 cytotoxic lineage. However, upon activating of T-cells by PHA and subsequent growth with IL 2, c-rel expression was induced to levels approaching those seen in B cells. This IL 2 induced increase in c-rel mRNA was transiently observed from 24-72 after induction and then returned to basal levels. In addition, an antisera made to portions of the v-rel transforming protein detected an 82,000 m.w. human rel-related proteins in cells possessing high levels of c-rel RNA.

III. Cytotoxic Effector Mechanisms are Active Against HIV-I and HTLV-I Infected Cells

AIDS is associated viral (HIV-I) mediated progressive depletion of a helper T4 T-T-cell subset, while ATL is associated with a viral (HTLV-I) mediated growth of the same T-cell subset. Since large granular lymphocytes (LGL) with natural killer (NK) activity have been shown to spontaneously lyse several virus infected target cells, the ability of NK cells to lyse both HTLV-I and HIV-I infected lymphoid cell lines and fresh lymphocytes was explored. Normal lymphocytes (T cells and LGL), with and without pretreatment with recombinant IL 2 as well as monocytes, with and without pretreatment with gamma interferon were employed as effectors. Both IL 2-activated T cells and NK cells were cytolytic for HTLV-1 infected targets. However, only LGL demonstrated significant spontaneous activity against HTLV-I infected targets. Similarly, LGL showed spontaneous cytolytic activity against HIV-I infected targets and this cytotoxicity was considerably augmented by IL 2. In contrast, T cells and monocytes were unable to lyse HIV-I targets and only minimal activity was induced by activation. LGL cells, B cells, and monocytes could be infected in vitro by high titers of HIV-I. However, levels of reverse transcriptase found in these cultures were significantly lower than the levels in T cell cultures. In contrast, only T-cells were susceptible to infection by HTLV-I. These results suggest that cellular immunity may play a role in host defense against these viral infections. Preliminary studies suggest that NK function will synergize with lower drug doses in blocking HIV replication.

IV. Neuropeptide Receptor-like Pattern of CD4 Antigen in Brain

The differentiation antigen T4 is present on the helper/inducer T cell subset, which is responsible for the humoral regulation of immune reactivity and hematopoietic development. In AIDS, this lymphocyte subset is selectively depleted by infection with HIV-I virus which apparently uses the T4 antigen complex on the T-cell surface as a viral receptor. Autoradiographic visualization of the T4 antigen on thin coronal sections of monkey and human brain reveals a strikingly heterogenous pattern with clustering in the same emotion-mediating nodal points of the brain that are usually enriched with neuropeptide receptors. Immunoprecipitates of radioiodinated cell membranes prepared from primate brain indicate that an antigen very similar to T4 antigen is present on brain as well as T4+ T-lymphocytes. Other T lymphocyte-associated antigens such as T8 were not found in the brain. Since patients with AIDS frequently develop complications of the central nervous system and HIV-I sequences have been found in the brain, these data suggest that direct infection of some brain cells with HTLV-I occurs via a mechanism involving the T4 antigen complex similar to that proposed for lymphocyte infection. In view of the brain sites found to express the T4-like antigen, the behavioral changes and mood shifts observed in AIDS patients may result from localized viral replication.

V. cis-Acting Transcriptional Regulatory Sequences in the Gibbon Ape Leukemia Virus (GALV) Long Terminal Repeat

Gibbon ape leukemia viruses (GALV) are a group of retroviruses which have been associated with hematopoietic neoplasms in primates. Two of the viruses, GALV-SEATO and GALV-San Francisco (GALV-SF), are associated with myeloid and lymphocytic leukemias, respectively, in apes. Using an assay based on the transient expression of the bacterial gene chloramphenicol acetyltransferase (CAT), we examined the transcriptional activity of GALV-SEATO and GALV-SF. The results suggest that high level expression of GALV is due primarily to cis-acting enhancer sequences. Sequence delineation analysis of GALV-SEATO showed the GALV-SEATO enhancer sequences to be located with a 45-bp tandem repeat in GALV-SEATO, GALV-SF, which has two-to five-fold transcriptional activity, contains only a single copy of the 45-bp element with a 6-bp difference from those in the GALV-SEATO enhancer element. The 45-bp element is highly homologous to sequences within the LTRs of several murine leukemia viruses but has not been examined for enhancer function in these retroviruses. High expression of GALV was restricted to hematopoietic cells and MLA 144 cells, a gibbon ape T-cell line known to be infected with GALV-SF. However, expression of constructs containing the CAT gene directed by GALV SEATO LTR sequences was similar in uninfected and GALV-infected fibroblasts, indicating the lack of virally encoded or virally induced trans-activating factors capable of increasing expression in these cells. Also, factors capable of trans-acting the

GALV-LTR were found in human myeloid and erythroid cells not infected by GALV, indicating that host factors selectively expressing in cell lineages can stimulate retroviral transcription.

VI. Interaction of Human Cytomegalovirus with HTLV and HIV-infected Lymphocytes

Amongst the other human viruses also know to infect lymphoid cells are some members of the Herpes virus family, particularly human cytomegalovirus (HCMV). This virus is also known to cause immunosuppression, and is commonly associated with AIDS-related deaths. Nevertheless, any interaction between HCMV and HTLV or HIV remains obscure, nor is it known whether the viruses can replicate simultaneously in the same cell. This present work was undertaken to investigate whether HCMV could infect T lymphocytes harboring the HTLV-I or HIV-I genome, and whether such superinfection with HCMV would result in an additive effect on any of characteristics of the host lymphocyte or the replication of either virus. Following challenge of HTLV I or II-infected lymphocytes with HCMV, an increase in HCMV titer was seen which peaked at 5 days post-infection, and infectious progeny virus could still be detected between 11 and 14 days post-infection. Similarly, in HUT 78 cells, a lymphoblastoid line from a patient with Sezary syndrome, HCMV was detected up to 11 days post-infection; however, when the same cell line was infected with HIV followed by challenge with HCMV, no infectious progeny could be detected after 5 days of virus challenge, suggesting that pre-infection with HIV interferes with the persistence of HCMV in these cells. Although 5-20% of cells expressed HCMV-specific antigen as detected by staining with HCMV-specific peroxidaselabeled antibodies, only 0.01-4% of the total cell population formed infectious centers when assayed on permissive fibroblasts. In HTLV I and HTLV II-infected cell lines, neither cell viability and proliferation nor were there any significant differences in the biological functions or surface markers followed infection by HCMV. In contrast, both HIV-infected and noninfected HUT 78 cells showed increased Tac expression 5 days after challenge with HCMV. HCMV did not induce the production of IL 2 or IFN-γ in any of the cell lines. Dot blot hybridization using a 32P-labeled probe to the immediate-early region of HCMV detected the expression of viral mRNA in all cell lines except for HIV-infected cells at 14 days post-infection, again suggesting that the presence of HIV interferes with HCMV persistence. To investigate the interaction between HTLV-I and HCMV, MT-2 cells were infected with HCMV and transfected with a chimeric plasmid construct consisting of the long terminal repeat (LTR) sequences derived from a molecular clone of HTLV-I fused to a bacterial chloramphenicol acetyltransferase gene (CAT). In contrast to the findings of others, we observed enhancement of the HIV-LTR directed expression of CAT by HCMV. These results demonstrate that HCMV can indeed replicate in a small proportion of lymphocytes previously infected with human retroviruses, and that for HTLV-I at least, transactivation of the HTLV-1 genome by HCMV can occur. The same kind of studies have been published showing HCMV transactivation of HIV-I LTR in HIV infected cells. This project has been terminated.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09254-06 LMI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemical and Molecular Mechanisms of Growth Factor Modulated Proliferation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. L. Farrar, Jr.	Senior Investigator	LMI, NCI
Others:	A. H. Bellan	Visiting Fellow	LMI, NCI
	A. T. Brini	Visiting Fellow	LMI, NCI

COOPERATING UNITS (if any)
National Institute of Mental Health, NIH (J. Hill); Division of Cancer Etiology, NCI (J. Cleveland); PRI, NCI-FCRF (L. Arthur).

LAB/BRANCH
Laboratory of Molecular Immunoregulation

SECTION
Lymphokine Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

IL 2 regulates the transcription of a number of genes including the proto-oncogenes, c-fos, c-myc, c-myb as well as other genes such as ornithine decarboxylase (ODC) and the IL 2 receptor α chain (Tac). Antiproliferative signals such as cyclic AMP selectively inhibit IL 2 directed increases in c-myc and ODC mRNA accumulation. The specific deletion of c-myc protein biosynthesis by synthetic "anti-sense" oligonucleotides inhibited PHA or IL 2 directed proliferation of human T cells. IL 2 also stimulates mRNA expression and protein synthesis of ancient stress genes such as HSP-70. We have investigated the DNA binding proteins which recognize the enhancer regulatory element of the HSP-70 gene. We are likewise examining DNA binding proteins specific for the c-myc and IL 2 receptor enhancer sequences do determine what signals modulate the effects of these proteins on their respective gene transcription.

We have shown that human T lymphocytes synthesize pro-opiomelanocortin (POMC) mRNA in response to mitogenic stimuli. Both T cells and large granular lymphocytes (LGL) possess stereospecific opiate receptor and opiate-neuroendocrine hormones, such as β-endorphin, modulate the biological functions of these cells. Using radioligand autoradiography we have characterized IL 2 receptors in rat brain. The receptor for HIV was also observed in primate and human brain, approximately 57 kd, and mapping to areas distinct from IL 1 binding sites. We have studied the presence and distribution of cytokine mRNA in brain using in situ hybridization histochemistry. IL 1β mRNA was detected in brain areas closely overlapping where IL 1 receptors are found. Only IL 1β mRNA, and not IL 1α, was seen in rat and human brain. The processed mRNA was of a similar size as seen in endotoxin-treated monocytes. The colony stimulating factor, IL 3, mRNA was also found in neurons in discrete areas of brain. The IL 3 mRNA is not associated with blood borne cells and shows a unique localization pattern when compared with IL 1β mRNA.

PROJECT DESCRIPTION

PERSONNEL

William L. Farrar, Jr.	Senior Investigator	LS, LMI, NCI
Annick H. Bellan	Visiting Fellow	LS, LMI, NCI
Anna T. Brini	Visiting Fellow	LS, LMI, NCI

OBJECTIVES

The laboratory has developed research programs in two areas: 1) biochemical and molecular mechanisms of lymphoid and myeloid growth, and 2) receptors and chemical signals common to the immune and central nervous systems.

MAJOR FINDINGS

I. Biochemical and Molecular Mechanisms of Lymphoid and Myeloid Growth

The identification of protein kinase activation and the cellular substrates of the enzymes is one of the most significant areas of growth factor and oncogene research.

We have recently initiated studies which are designed to examine the participation of tyrosine kinases in lymphokine-mediated signal transduction. Previous studies used techniques which detected the most abundant phosphorylation reactions, namely serine-threonine. We have now employed anti-phospho-tyrosine affinity capture methods using aby-linked sepharose beads to enrich for substrates phosphorylated on tyrosine residues. We have found that IL 3 stimulates tyrosine phosphorylation of a number of proteins including a 140 kd protein that we suspect is associated with the receptor. Interestingly, phorbol ester also activates tyrosine phosphorylation of these substrates again implying that interkinase systems are regulated by IL 3 and other growth factors.

Now that certain molecular loci have been identified for the action of IL 2 and some of the CSFs, we can examine the effects of anti-proliferative agents on these genes. We have shown that the lymphocytes anti-proliferative signal cyclic AMP, inhibits IL 2 stimulate c-*myc* and ODC mRNA accumulation. This is not a general effect since cAMP induced c-*myb*, c-*fos* and Tac mRNA synthesis. cAMP, however, suppress IL 2 mRNA synthesis. We are currently examining the effects of cAMP on the regulatory regions of three genes affected by it. Using regulatory region-CAT reporter gene constructs for IL 2-CAT, IL 2 recpt.-CAT, and c-*myc*-CAT, we are determining 1) whether a cAMP responding region exists within these regulatory regions; 2) the participation of cAMP-dependent kinase and 3) using in vitro transcription, how a negative signal effects enhancer binding proteins or otherwise regulates transcription. We have deduced a sequence element which responds to cAMP activation in T lymphocytes. We intend to purify and sequence this protein and determine how it functions to modulate gene transcription of cAMP regulated genes (i.e., c-myc, Tac and IL 2). These studies will be similar to those discussed above with the intent to understand the biochemical basis of negative gene regulation.

The effects of IFN-gamma on gene regulation stimulated by CSFs was examined. Harel-Bellan et al. has shown that IFN-gamma did not inhibit c-myc gene transcription but c-myc mRNA accumulation was severely impaired. The studies demonstrated that IFN-gamma inhibited the maturation of nuclear precursor c-myc RNA into mature mRNA. IFN-gamma treatment of CSF-dep. myeloid cells inhibited the intron splicing process unique to c-myc precursor RNA. On the other hand, IFN-gamma inhibited the transcription of another proliferation associated gene, ODC, as does cAMP in lymphoid and myeloid cells. These studies have allowed us to examine the points of molecular inhibition that specific anti-growth signals act upon. We intend to examine these phenomenon in responsive and unresponsive malignant homologous cell lines.

Progression of cell cycle or terminal differentiation has been associated with the expression of new genes which provide additional de novo instructions to the cells. We have examined a modest number of genes expressed by IL 2 stimulation of G_1 arrested T lymphocytes. Among these implicated in cell cycle progression for tissues or diverse origins are the nuclear proto-oncogenes. While many of the cytosolic or membrane bound proto-oncogenes (some with kinase activity) mRNA are upregulated in G_o -> G_1, the nuclear proto-oncogenes c-fos, c-myc, and c-myb are sequentially regulated by IL 2. Cleveland et al. showed that IL 2 stimulates the transcription and steady state mRNA accumulation of these nuclear proto-oncogenes and not cytosolic/membrane oncogenes. Subsequent studies by Harel-Bellan and Farrar have also shown that IL 3, G-CSF and GM-CSF stimulate essentially the same program of expression. Phorbol ester also triggers much of the same gene expression as the growth ligand in either lymphoid or myeloid cells. Other genes such as ornithine decarboxylase (ODC) important for cellular proliferation are also stimulated by IL 2 or CSFs. Recently, we have identified that an extraordinarily ancient set of genes are regulated by IL 2 or CSFs. Ferris et al. have shown that IL 2 stimulates steady state mRNA and protein synthesis of a member of the heat shock protein family, HSP 70. Similarly, we have found that IL 3, GM-CSF and G-CSF regulate the expression of these proteins in myeloid cells.

One method employed to specifically delete gene products believed to be involved in the regulation of biological responses to exogenous stimuli has been "anti-sense" oligonucleotides. We have used short (15 mer) oligonucleotides complementary to the second exon of c-myc to evaluate the effects on c-myc protein synthesis and mitogen induced T cell proliferation. Harel-Bellan et al. showed that anti-sense c-myc oligos inhibited c-myc protein synthesis in PHA-stimulated human T lymphocytes. The deletion of c-myc protein synthesis abrogated mitogen or IL 2 induced proliferation. We have recently employed this strategy to delete IL 2 or IL 4 lymphokine expression in autocrine helper T cell clones, proving this technique applicable to the deletion of intracellular proteins and secretory molecules. We intend to test these procedures against other gene products known to upregulate during the IL 2 directed cell cycle. Mandatory for this approach is the ability to directly detect the protein of interest. We are currently examining oligonucleotide inhibition of the c-myb, cyclin and p53 onc gene products. Selective deletion of such gene products will allow us to determine their obligatory expression for cellular proliferation, but more important, subsequent gene expression or biochemical events "downstream" from the synthesis of the protein of interest. For instance, we are examining whether c-myc deletion inhibits the activation of DNA polymerase α. We also propose to introduce maby to selective proteins (i.e. c-myc, c-myb, cyclin) by electroporation in order to neutralize protein function. Preliminary experiments have suggested that electroporated maby function as efficiently as microinjected maby studies but without the limitations of standard microinjection procedures.

II. Common Cytokines to the Immune and Central Nervous Systems

Utilizing molecular probes we have addressed whether the brain can produce lymphokine-like ligands which perhaps effect a biological role in hybridization histochemistry in a fashion analogous to the labeled protein ligand interactions. In this procedure, the labeled cDNA hybridized with its complementary mRNA in a highly specific manner. We have examined rat and mouse brain for the present of IL 1β mRNA and the hematopoietic growth factor, interleukin 3 (IL 3) mRNA.

In situ hybridization histochemistry detected IL 1β mRNA throughout the rat brain in a pattern closely resembling the distribution of ^{125}I-IL 1 binding. Hybridization of IL 1α cDNA was undetectable. Cells containing IL 1β mRNA were particularly prominent in the hippocampus. IL 1β mRNA was detected in the granule cell layer of hippocampus, suggesting that it is synthesized in neurons. Northern analyses of RNA from rat brain did not reveal IL 1α cDNA hybridization but only IL 1β cDNA hybridization. Principal RNA hybridization species to IL 1β cDNA corresponded to those seen in monocytes.

IL 3 mRNA was detectable in cells through mouse brain with the densest accumulation of IL 3 mRNA-positive cells in the medial habenulae. Cell dense regions in the hippocampus and cerebellum were also enriched with IL 3 mRNA-containing cells. IL 3 cDNA hybridized with mRNA of a size characteristic of the murine myeloid cell line WEHI-3 evaluated by Northern analysis.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09264-06 LMI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Normal and Neoplastic Hematopoietic Cell Growth: Role of BRMs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. W. Ruscetti	Senior Investigator	LMI, NCI
Others:	M. C. Birchenall-Sparks	Guest Researcher	LMI, NCI
	N. C. Lohrey	Microbiologist	LMI, NCI
	G. K. Sing	Visiting Fellow	LMI, NCI

COOPERATING UNITS (if any)

LEI, BRMP, DCT, NCI (J. Ortaldo, R. Wiltrout); LMI, BRMP, DCT, NCI (H. Young, G. Varesio); OAD, BRMP, DCT, NCI (D. Longo); PRI, NCI-FCRF (W. Urba, J. Rossio, J. Keller, K. Sill, P. Hewetson).

LAB/BRANCH

Laboratory of Molecular Immunoregulation

SECTION

Lymphokine Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In studying negative regulation of hematopoietic cell growth, we have found that transforming growth factor β is a potent inhibitor. TGF-β1 and TGF-β2 are equipotent inhibitors which have a selective effect on hematopoiesis by halting the growth of only early human and murine progenitors. By using immature murine hematopoietic cells that respond to IL 3, it was shown that TGF-β acted directly on the cells to block multipotent cell development but not unipotent cell growth. The earliest measurable events in vitro such as high proliferative potential colony formation (HPP-CFU) and induction of Thy-1 antigen were also inhibited by TGF-β. In contrast, there was no effect on G-CSF stimulation of granulopoiesis. In euthyroid development, CFU-GEMM and BFU-E but not CFU-E were inhibited. Thus, the ability of TGF-β to block hematopoietic progenitor cell growth depends on the differentiated state of the cell and suggests that TGF-β is an important regulator of hematopoiesis. Fresh AML and CML as well as early myeloid leukemic cell lines retained sensitivity to positive stimulation of CSFs and negative inhibition by TGF-β. The lone exception was HL-60, a human promyelocytic cell line, which was found not to have receptors for TGF-β. However, agents that stimulate differentiation of HL-60 caused a reappearance of the TGF-β receptors on the cell surface. These results suggest that the unresponsiveness to agents can be overcome in leukemic cells. In contrast to myeloid leukemic cells, cultured lymphoid leukemic cells were insensitive to TGF-β inhibition, whereas normal lymphoid cells are sensitive to TGF-β. Thus, this loss of negative regulation may play a role in the growth of some leukemic and malignant lymphoid cells.

PROJECT DESCRIPTION

PERSONNEL

Francis W. Ruscetti	Senior Investigator	LS, LMI, NCI
Maria C. Birchenall-Sparks	Guest Researcher	LS, LMI, NCI
Nancy C. Lohrey	Microbiologist	LS, LMI, NCI
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MAJOR FINDINGS

I. Transforming Growth Factor: A Selective Growth Inhibitor for Hematopoietic Progenitor Cells

In general, two techniques have been used to study hematopoietic cell growth and differentiation in vitro: (1) the measurement of cell number and type in suspension culture and (2) colony formation by different cell types in semi-solid media. Both assays depend on adding exogenous humoral factors for growth stimulation. Factors used here include recombinant murine GM-CSF, recombinant human G-CSF and CSF-1 (M-CSF), which induce terminal maturation of the cell types for which they are named, and erythropoietin (Epo), which induces end-stage erythroid maturation. Also, IL 3 which induces multipotential bone marrow colony formation (CFU-GEMM) consisting of granulocytes, erythroid cells, monocytes and megakaryocytes, was used. TGF-β1 is a potent inhibitor of IL 3 induced murine bone marrow proliferation and colony formation but, surprisingly, has little or no effect on the growth and differentiation induced by GM-CSF, G-CSF or CSF-1. TGF-β1 only inhibits early erythroid differentiation which is stimulated by Epo in combination with other factors in the GEMM or BFU-E assay while Epo-induced terminal erythroid differentiation (CFU-E assay) is unaffected. Also, IL 3 but not GM-CSF induced granulocyte-macrophage colonies were inhibited by IL 3. However, small clusters (5-20 cells) of terminally differentiated pure myeloid colonies were consistently seen in cultures containing IL 3 and TGF-β1. These results suggest that TGF-β1 selectively inhibits early murine hematopoietic progenitor growth and differentiation but not more mature progenitors. These inhibitory effects of TGF-β are at least partially reversible and non-toxic, since cell viability was not effected and colony formation was restored by 50% by washing TGF-β1 out after 48 hrs. Antisera to the N-terminus of TGF-β1 completely abrogated the inhibitory effects of TGF-β1 on hematopoiesis but had no effect on the identical inhibitory effects of TGF-β2.

We next sought to extend these observations on the effect of TGF-β to the proliferation of normal human bone marrow. In contrast to the mouse, both human IL 3 and GM-CSF, in the presence of Epo, promoted the growth and differentiation of multipotent as well as erythroid BFU-E colonies in vitro. In the presence of TGF-β1, only pure small clusters of either differentiated granulocytes or monocytes were observed. Therefore, TGF-β1 blocks the growth and differentiation of early human hematopoietic progenitors while allowing clusters (less than 40 cells) of more differentiated progeny to develop. Also, GM colony formation induced by IL 3 and GM-CSF was inhibited by TGF-β1. However, as in the mouse, G-CSF induced colony formation was not inhibited by TGF-β. Removal of T-cells, monocytes and other adherent cells from bone marrow did not alter these results suggesting TGF-β1 was acting directly on the target cell. In our hands, TGF-β1 and TGF-β2 had identical effects on inhibiting hematopoietic cell growth and colony formation at similar doses in all these assays. Therefore, TGF-β1 and TGF-β2 suppress the growth and differentiation of early growth factor-mediated events but are inactive on late acting growth factor-mediated myeloid and erythroid responses.

II. Primitive Hematopoietic Progenitor Cell Growth: Effect of TGF-β

Stem cells surviving a single injection of 5-fluorouracil are more primitive than the average hematopoietic stem cell. An assay has been developed to measure this high proliferative potential colony forming cell (HPP-CFU). The assay requires a synergistic activity (IL 1) which acts on these cells enabling them to respond to either CSF-1 or IL 3, probably by stimulating increase of specific receptors. The expansion of either of these stem cells with HPP-CFU potential is inhibited by TGF-β. In addition, Thy-1 negative cells must express Thy-1 positivity induced by IL 3 before these cells can form colonies of any lineage in response to IL 3. TGF-β inhibits the ability of Thy-1 negative cells to become positive. If the cells are allowed to become Thy-1 positive and then purified by sorting on a fluorescence activated cell sorter, IL 3 can then induce CFU-GEMM and CFU-GM colonies which are inhibited by TGF-β whereas formation of CFU-M and CFU-G colonies are not inhibited. In addition to showing that TGF-β inhibits the earliest hematopoietic events measurable in vitro, these results suggest that action of TGF-β is a direct one.

III. Regulation of Hematopoietic Tumor Cells by Transforming Growth Factor β

Since escape from the action of negative regulators such as TGF-β could play an important role in the growth of malignant cells, we then examined the effects of TGF-β on myeloid and lymphoid leukemic cells. As previously discussed, TGF-β acts as an important negative modulator lymphoid cell growth. Surprisingly, it was not inhibitory for the growth of any of mature human T and B lymphoma cell lines or virally-transformed T cell lines. In a small number of fresh lymphoma cells, some were inhibited and others were not. These results suggested escape from negative regulation could play a role leukemic lymphoid cell growth. However, all the myeloid cell lines with the exception of HL-60 that were examined remained sensitive to TGF-β.

Since cell lines growing continuously in culture may not be truly representative of freshly isolated malignant cells, we examined the effect of TGF-β on fresh tumor specimens. In general, growth factor responsive leukemic cell progenitors from patients with acute (AML) and chronic (CML) myelogenous leukemia were approximately 5-10 fold more sensitive to inhibition by TGF-β than cells from normal donors. Nevertheless, a small population of these cells remains insensitive to TGF-β inhibition, since the degree of proliferation at maximum inhibition is still above that of background inhibition in the absence of colony stimulating factor. Unlike normal BM, the G-CSF induced proliferation of CML and AML cells was also sensitive to TGF-β inhibition. To further study the effects of TGF-β on leukemic cell populations, number of cell lines of different lineages were tested for sensitivity to TGF-β. In all cases tested, TGF-β1 inhibits the growth of IL 3 induced murine myeloid cell lines such as FDC-P1 and DA-1, regardless of their derivation. The effective dose that resulted in 50% of maximal stimulation (ED-50) was similar for all cell lines. In addition to responding to IL 3, one cell line, NSF-60, proliferates in response to GM-CSF, CSF-1, G-CSF, IL 4 and IL 6. Growth factor-induced proliferation of NFS-60 is also inhibited by TGF-β1 in a dose-dependent manner with ED-50's of 6-10 pM. Since normal bone marrow cell growth and differentiation stimulated by GM-CSF and G-CSF were not inhibited by TGF-β, these results with NSF-60 suggest that the state of differentiation of a cell and not the growth factor responsiveness determines whether TGF-β will have an inhibitory effect. Human cell lines of both myeloid and monocytic were sensitive to TGF inhibition, although there were also lines such as HL-60 and K562 which were insensitive.

To attempt to define the difference between those leukemic cell lines that respond to TGF-β and those did not, possible differences in TGF-β receptors were studied on two human myeloid leukemic cell lines, KG-1 (myeloblasts) is inhibited by TGF-β while HL-60 (promyelocytes) is not. Specific binding with ^{125}I-TGF-β1 and β2 was observed for KG-1 but not for HL-60. Cross-linking studies using ^{125}I-TGF-β1 and β2, showed that KG-1 expresses the previously described 200-280 Kd, 85 Kd and 65 Kd of TGF-β receptor. However, when HL-60 was made to differentiate in the presence of PMA or DMSO, the TGF-β was then present on the cell surface. These results suggest that some cells may continuously grow as a result of loss of receptors for negative regulation by TGF-β.

IV. Expression of the Nuclear Proto-oncogenes (c-fos, c-myb and c-myc) in Hematopoietic and Lymphoid Cell Growth: Effect of TGF-β

Transcriptional activation of the cellular proto-oncogenes (c-onc), by IL 2 and IL 3 was studied in the responsive murine T-lymphocyte clone, CT6, and murine myeloid cell line, FDC-P1, respectively by nuclear run off transcription assays. Both growth factors induced a sequential increase in c-fos, c-myc, and c-myb mRNA synthesis. PMA, a direct activator of protein kinase C (PK-C), also resulted in a similar temporal induction in mRNA synthesis for the same c-onc genes. An increase in the accumulation of the c-fos, c-myc, and c-myb mRNAs was confirmed by Northern blot analysis. These results indicate that PMA and lymphokine growth factors regulate early c-onc gene transcription by similar biochemical pathways. Since both phorbol ester and interleukins activate PK-C, this phosphotransferase system appears to be an important pathway in gene activation by these agents. However, phorbol esters also stimulate transcription of these proto-oncogenes but not proliferation in these cells, suggesting that the common biochemical events shared by PMA and interleukins do not alone account for the mitogenic effects. Preliminary studies on mechanism of growth inhibition by TGF-β has shown that IL 2 stimulation of c-myc mRNA transcription is not effected by TGF-β. Studies will now be done to assess stability and translation of these mRNAs. In addition, the role of the c-oncs (myc, myb and fos), encoding for nuclear proteins was evaluated during the induction of myeloid cell differentiation using the Wehi described above. C-myc, c-myb and c-fos mRNA levels were measured in both Wehi 3B cells positive and negative for differentiation induction following treatment with PMA and vitamin D3. Our results showed that although PMA did not induce phenotypic differentiation of the Wehi-3BD- cells, modulation of proto-oncogenes was seen. PMA induced a reduced expression of c-myc and c-myb and stimulated significant expression of c-fos. On the other hand, vitamin D3, which did induce phenotypic differentiation of the Wehi-3BD-cells showed the same changes. Wehi 3BD+ cells which differentiate into macrophages in response to PMA, but not vitamin D3, showed the same small changes in c-myc, c-myb and c-fos expression to the inducer. Thus, modulation of expression of these genes does not correlate with the ability of the cells to be induced to differentiate. The effect of TGF-β on the ability of these cells to differentiate and modulate c-onc expression is being studied.

V. Effect of v-abl on Leukemic Myeloid Growth and Differentiation

The myelogenous leukemic cell line NFS-60 is an hematopoietic progenitor blocked in differentiation. Its growth factor requirements can be met by a number of hematopoietic growth factors. Another cell line, 32D CL-23, required IL 3 for its growth. These two cell lines were infected with Abelson murine leukemia virus (A-MuLV) containing the onc gene v-abl. Subsequent selection of A-MuLV-infected NFS-60 and 32D CL-23 in growth factor-free medium resulted in the establishment of growth factor independent clones (NFS-60-ABL's, 32-DC-ABL's). The abrogation of factor requirement by v-abl has been demonstrated in other cells. These clones expressed abl specific message by Northern analysis as well as the p120 gag-abl fusion protein. Although v-abl led to the abrogation of factor dependence in both cell lines, marked differences were observed in the ability of these two lines to undergo differentiation. Specifically, NFS-60-ABL's spontaneously differentiated into morphologically identifiable macrophages at various stages in differentiation with cells containing irregularly shaped nuclei and lightly stained vacuolated cytoplasm, and a high cytoplasm to nuclear ratio. In comparison, the parental NFS-60 contained immature myeloblasts with scant cytoplasm and oval nuclei. The introduction of v-abl into NFS-60 has partially overcome the block in differentiation in addition to abrogating factor dependence. In contrast, 32D CL-23-ABL, does not undergo spontaneous differentiation when it loses its dependence on the factor. These observations suggest that in addition to the abrogation of factor dependence, expression of v-abl at certain stages of hematopoietic development promotes differentiation down the monocytic lineage. In addition to these results, NFS-60-ABL exhibit subsequent non-specific esterase staining (macrophages), become positive for MAC-1 (macrophages), and show decreased levels of Thy-1 expression-associated with differentiation, as well as increased levels of Fc receptor expression based on Facs analysis. Thus, the gene product of v-abl does not block cells in certain stages of differentiation but can induce further differentiation. To better define role of the Abl protein in myeloid differentiation, cells will be constructed with a temperature-sensitive mutant of v-Abl. With these cell lines, one could study the comparative effects of the v-Abl on differentiation at two distinct stages of differentiation.

The ability of v-abl, v-src, v-fms to abrogate growth factor dependence of these cell lines was a constant finding. Surprisingly, the action of these three oncogenes did not effect the ability of TGF-β to inhibit the growth of these cells. In fact, these cells containing these oncogenes were slightly more sensitive to TGF-β inhibition suggesting that other events are necessary to overcome negative regulation of these cells.

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SUMMARY REPORT

LABORATORY OF EXPERIMENTAL IMMUNOLOGY

October 1, 1987 to September 30, 1988

INTRODUCTION

The Laboratory of Experimental Immunology (LEI) of the Biological Response Modifiers Program (BRMP), DCT was formed in 1985 as a result of the reorganization of the existing Biological Therapeutics Branch (BTB), with Dr. John R. Ortaldo as the Laboratory Chief. The LEI is composed of three sections: the Cellular and Molecular Immunology Section, Dr. Craig W. Reynolds, Head; the Leukocyte Differentiation Section, Dr. John R. Ortaldo, Acting Head; and the Experimental Therapeutics Section, Dr. Robert H. Wiltrout, Head.

The LEI conducts studies on biological response modification and the application of these studies to the therapy of cancer. Indepth studies are performed on cell-mediated immune effector mechanisms, lymphokines, monoclonal antibodies (MoAb), growth factors, and other host responses that may be useful for cancer treatment. Selected biological response modifiers (BRMs) are studied for their effects on the immune system and other aspects of host responses, with a particular focus on the therapeutic implications of such effects. Based on such information, protocols for therapy of tumors in experimental animals and in cancer patients are developed and studies are performed to evaluate the therapeutic efficacy of selected BRMs.

CELLULAR AND MOLECULAR IMMUNOLOGY SECTION

The Cellular and Molecular Immunology Section (CMIS; Dr. Craig Reynolds, Head) studies the role of the cellular immune system in mediating antitumor immune responses. The general goals of this section are to use molecular approaches and a basic knowledge of the cellular immune system 1) to investigate in detail the ability of BRMs to augment and regulate natural and acquired antitumor immune responses; 2) to study the cellular and molecular mechanism(s) by which these antitumor effector cells mediate their activity; and 3) to study the mechanism(s) by which tumor cells escape these biological and immunological defense systems. The specific aims of the CMIS are 1) to conduct basic and applied research regarding the natural immune system in humans and experimental animals; 2) to study human and murine specific T cell-mediated immunity, with emphasis on T cell surface molecules and mechanisms of tumor cell lysis; 3) to study the control of lymphocyte activation; and 4) to study the mechanisms of tumor cell resistance, including resistance to chemotherapeutic and immunological effector molecules.

The CMIS devotes considerable attention to the study of the recognition and post-recognition events of tumor cell lysis by CD3- LGLs. This lytic event can be divided into three distinct stages: 1) target cell recognition and binding, 2) release of cytolytic factor(s) and 3) target cell lysis. Although the mechanism by which NK cells recognize tumor cells eluded identification, our studies have considerably enhanced the understanding of this event. Molecular analysis of the T cell receptor genes has shown that functionally active human and rat LGLs lack α-, β- and γ-chain rearrangement or mRNA expression. Therefore, we know that, unlike cytotoxic T cells, the T cell receptor is not involved in the recognition of target cells by LGLs. Furthermore, analyses of the methylation state of the T cell receptor β-chain gene have demonstrated that this gene is predominantly unmethylated in T cells, highly methylated in B cells and monocytes and intermediately methylated in LGLs. These results indicate that the methylation pattern of the T cell receptor β-chain gene can be used as a marker for T cells and LGLs.

While studying target antigens on K562 cells, the CMIS developed a MoAb that blocks the binding and lysis of CD3- LGLs. An anti-idiotypic antibody has also been developed, with the idea that it would recognize the NK antigen receptor (NK-R) and facilitate its identification. This antibody reacts with an 80 kD protein and, consistent with the hypothesis that the MoAb will recognize the NK-R, F(ab')2 fragments of the antibody 1) react primarily with CD3- LGLs, 2) block binding and lytic functions and 3) induce significant activation of LGL effector functions after pretreatment. The CMIS is working to produce a MoAb to this 80 kD molecule and toward the further biochemical characterization and cloning of the NK-R.

The actual mechanism of LGL cytotoxicity has been explored using the rat LGL leukemia system. These studies have demonstrated that purified cytoplasmic granules and an associated 60 kD protein (cytolysin) may mediate much of the cytotoxic activity of LGLs. These results provide the first direct evidence that a secretory event involving these granules is associated with the lysis of both tumor cells and microbial agents. The observation that LGLs secrete numerous soluble cytotoxic factors [e.g., tumor necrosis factor alpha (TNFα), IFNα/γ] after contact with NK-susceptible target cells provides support for the notion that NK cytotoxic activity involves a secretory event. The CMIS has particularly focused on the specificity and mechanism of action of NK cytotoxic factor (NKCF). NKCF has been compared, in terms of its production and properties, to other recombinant cytokines, including lymphotoxin (LT) and TNF. The CMIS has found that NKCF is distinct from these cytokines, because antibodies directed to IFNα, IFNγ, LT or TNF failed to neutralize the cytotoxic activity observed with NKCF preparations. To assist in the characterization and purification of NKCF, mouse MoAbs that neutralize the NKCF-related cytolytic activity of rat and human LGL-derived supernatants have been developed by the CMIS. It is of interest that these MoAbs do not inhibit TNFα, IFNα/γ, LT or leukoregulin. The importance of the NKCF molecule in cell-mediated cytotoxicity was suggested by the observation that it inhibits NK activity in rat cells treated with these MoAbs. Biochemical analysis of labeled NKCF-containing supernatants indicated that the major protein recognized by these anti-NKCF MoAbs is approximately 12,000 kD. These anti-NKCF MoAbs should be very useful in further purifying and biochemically characterizing NKCF and for studying its role in various cell-mediated cytotoxicity assays.

In addition to their studies with NKCF, the CMIS has reported that CD3- LGLs produce and secrete various other cytokines, including IL1α/β, IL2, IFNs, colony stimulating factor (CSF) and B cell growth factor (B cell growth factor: IL4). These findings have stimulated interest in the NK cell as a potent immunoregulatory cell. In collaboration with Dr. Young (LMI), the CMIS has demonstrated that IL2, in addition to its role as a potent activator of cytotoxicity, induces transcription/translation of IFNγ from purified populations of CD3- LGLs. To initiate IFNγ production, CD3-/CD16+ LGLs require only a single signal with a lymphocyte-activating agent such as phytohemagglutinin, phorbol myristate acetate, IL2 or ionomycin. In contrast, CD3+ T cells require two stimuli for high levels of IFNγ production. Present studies are being extended to examine the role of IL1, IL4, macrophage CSF and other biologically relevant cytokines in stimulating IFN production.

Another major focus in the CMIS has been to study the regulation of human and murine NK activity. IL2 treatment of LGLs dramatically broadens target cell specificity and strikingly augments the overall degree of lysis. On the basis of IL2's role as a potent activator of cytotoxicity against fresh autologous tumor target cells, studies have been initiated to examine the progenitor and effector phenotypes of these lymphokine- activated killer (LAK) cells. In humans, it is evident that several lymphoid subsets of peripheral blood mononuclear cells, including CD3+/CD16- and CD3-/CD16+ lymphocytes, can give rise to and mediate LAK cell activity against fresh tumor cells. However, the CD3- LGL population is the largest single group of precursor cells capable of generating LAK activity from human and rat peripheral blood. The observation that IL2 treatment of LGLs alone increased NK activity, stimulated IFNγ secretion and promoted LGL growth in the absence of the TAC IL2 receptor fostered the hypothesis that LGLs express an IL2 receptor other than the TAC molecule. In support of this hypothesis, IL2 increased the lytic capacity of LGLs and the production of IFNγ in the presence of an anti-TAC (p55) MoAb. These observations contributed to the discovery of the β-chain (p75) of the IL2 receptor, constitutively expressed on CD3- LGLs. Further studies are under way to characterize the expression of the IL2 receptor β-chain and its regulation in LGLs.

Recent studies from the CMIS have also examined the in vivo relevance of LGLs. These studies have demonstrated that LGLs have several important antitumor effects in vivo. The adoptive transfer of LGLs into rats with depressed NK activity restored 1) in vitro cytotoxicity of peripheral blood lymphocytes, 2) in vivo clearance of tumor cells from the lungs and 3 resistance to the development of experimentally induced lung metastases. Additional studies have shown that NK cells can regulate the development of mature subpopulations of bone marrow (BM) cells and therefore affect engraftment in a BM transfer model. The potential of NK cells to regulate autologous and allogeneic BM engraftment is presently being examined in various BM transfer models.

Since CD3- LGLs mediate ADCC by means of their Fc receptors, studies have also examined the potential role of ADCC in antitumor responses. A series of murine MoAbs against NK-resistant melanoma and colon carcinoma cells were used to identify the peripheral blood effector cells that mediate ADCC against these targets. These studies demonstrated that, in humans, the cells mediating this activity have LGL morphology and phenotype. Additional studies revealed that the IgG3 MoAb functioned most efficiently in ADCC and that the ADCC effector cell was regulated in vitro by IFN and IL2, as described previously with regard to the augmentation of NK activity. Preclinical studies, using these MoAbs in combination with IFN or IL2, are under way in collaboration with Dr. Pearson (ETS, LEI).

To improve our understanding of the mechanism of target cell susceptibility/resistance, the CMIS also recently began studies to examine the mechanism(s) by which tumor cells escape immunological and drug-induced antitumor effects. Initially, we have studied glutathione S transferase (GST), a family of isozymes involved in drug metabolism. One approach to the problem is to produce MoAbs to GST and to use these MoAbs to examine GST expression in lysolecithin-permeabilized human tumor cells. We have found that a wide variety of human tumor cells express significant levels of GST. We plan to use the expertise and information gained from these GST studies to search for novel mechanisms of drug resistance in melanoma and colon and renal cancer models. A similar approach, involving the production of MoAbs and the transfection of relevant genes into sensitive cells, will be used to examine the inherent and acquired resistance of tumor cells to immunological effector mechanisms (i.e., TNFα, IFN, cytolysin, etc.).

LEUKOCYTE DIFFERENTIATION SECTION

The Leukocyte Differentiation Section (LDS; Dr. John Ortaldo, Acting Head) studies differentiation and activation of human and murine lymphocytes. The LDS also provides expertise for the selective development of new MoAbs required by investigators within the BRMP. The primary goal of the LDS is to use existing probes and to develop new probes for analyzing the differentiation and activation of hematopoietic cells. The focus of this group is to identify and manipulate immunologically important cell subsets in normal and disease states. Presently, the highest priority is to develop MoAbs that recognize cell surface receptors and detect the molecules involved in the activation or functional differentiation of leukocytes.

In the LDS, the differentiation schemes of various lymphocyte subsets from normal mouse thymus have been examined in vivo and in vitro. Experimental cell transfer studies have demonstrated that the most immature subset of adult mouse thymocytes is a dull Lyl+ (dLyl) cell that lacks both Ly-2 (CD8) and L3T4 (CD4). These dLyl cells contain committed thymocyte progenitors with a limited capacity for regeneration. Several more differentiated subsets have also been identified in the thymus. These include the Ly2+, L3T4+ blast population and the mature L3T4+ and Ly2+ subsets. Questions relating to the gene expression, growth factor receptors and proliferation potential of these subpopulations are being addressed.

The LDS has developed a model to further our understanding of the origin, differentiation and potential function of murine and human NK cells. In these studies, the LDS has compared CD3- LGLs isolated from mouse liver with the CD3- dLyl thymocyte subset. It is evident that i.v. injection of LGLs or BM will result in donor-derived LGLs, whereas only the BM successfully yields donor-derived cells in the liver after intrathymic transfer. Unexpectedly, studies have shown that the dLyl thymic subset can repopulate liver LGLs. Thus, the dLyl thymocyte appears to represent a population of cells that can regenerate liver LGLs as well as mature T cells. These studies are the first to link CD3- LGLs with a specific T lineage progenitor.

Until recently, only allelic NK-associated antibodies have been available to identify or study the lineage of murine NK cells. However, the LDS has generated a rat MoAb (4D11) that recognizes a novel 88 kD antigen (LGL-1) on the surface of LGLs isolated from mouse liver or spleen. Immunofluorescence studies indicate that this antigen is expressed on the LGLs of all strains of mice but is not detected on thymocytes, T or B lymphocytes or other hematopoietic populations. Further studies to examine the contribution of the LGL-1 lymphocyte subset to LAK or augmented NK activity are under way in the LDS.

To elucidate the role of oncogenes in cellular differentiation and malignant transformation, the LDS has generated murine MoAbs to the *fos* and *raf* oncogene products. Anti-*fos* MoAbs were developed using synthetic peptides representing two distinct molecular domains. One MoAb (2G9C3), raised against a 24 amino acid consensus region of both v-fos and c-fos, detects three proteins with molecular weights of 55,000, 44,000 and 42,000. As expected, immunohistochemical analysis of virus-infected cells revealed intense nuclear staining of fos, whereas no cell surface membrane expression was detected. Because evidence suggests that the ras oncogene, located on the cytoplasmic face of the cell membrane, may influence the expression of fos in the nucleus, the LDS, in conjunction with Drs. Carbone and Varesio (LMI), has begun to study this interaction in fibroblast cell lines transformed by the ras oncogene. Although fos protein can be induced in normal as well as N-ras- and K-ras-transformed fibroblast lines, the evidence suggests that it is constitutively expressed in H-ras-transformed lines.

The LDS also generated an anti-raf MoAb (MUR 1.4) against a 30 kD v-raf protein produced in Escherichia coli. Preliminary evidence suggests that MUR 1.4 immunoprecipitates c-raf from cells transfected with multiple copies of the raf gene. This antibody detects a 44 kD cytoplasmic antigen in the myc/raf transformed myelomonocytic cells. Studies are in progress in collaboration with Drs. Smith and Kung (Laboratory of Biochemical Physiology) to determine the effect of this MoAb on cell division and morphology following microinjection into adherent cell lines transformed with the raf oncogene. Using a combination of these MoAbs, it should be possible to investigate the involvement of oncogenes in tumorigenesis as well as to determine their location in the sequence of gene interactions leading to malignancy.

EXPERIMENTAL THERAPEUTICS SECTION

The Experimental Therapeutics Section (ETS; Dr. Robert H. Wiltrout, Head) provides a focus within the BRMP, rapidly translating the newest and most promising cellular and molecular biological observations into appropriate experimental therapy models in vivo. The overall goals of the section are 1) to determine the antitumor efficacy of BRMs and 2) to study the biological mechanisms by which these agents function.

The ETS is currently pursuing several areas of study. First, a major emphasis of the section has been to develop and utilize experimental models of chemoimmunotherapy (CIT) and adoptive chemoimmunotherapy (ACIT) for the treatment of both primary tumors and their metastases. To determine the effectiveness of CIT for tumors of various histological types located in different anatomical compartments, these studies are performed in models that produce tumors at various sites. In addition, the ETS investigates the efficacy of CIT and ACIT, with special emphasis on the nature of the responder and effector cells and the contribution of the recipient's immune system to observed therapeutic effects. The ETS has performed extensive studies to determine the therapeutic efficacy of adoptive immunotherapy (AIT) with recombinant IL2 (rIL2)-stimulated lymphocytes and/or exogenous rIL2 in combination with chemotherapeutic drugs for the treatment of murine renal cancer. It has been shown that AIT or chemotherapy with doxorubicin hydrochloride does not cure mice bearing Stage I renal cancer, while combination ACIT cured approximately 70% of these mice. Further studies have highlighted the need to localize AIT at all sites of tumor growth to ensure successful treatment of advanced (Stage III) murine renal cancer. More recent studies have demonstrated that the investigational drug FAA potently augments NK activity in spleen, liver, lungs and peritoneum. Since NK cells can serve as precursors for IL2-activated LAK cells, additional studies were performed to demonstrate that FAA and rIL2 have synergistic antitumor effects against murine renal cancer. Studies are in progress in the ETS to determine the mechanism(s) by which FAA augments NK activity and cooperates with rIL2. This type of approach may provide an experimental preclinical method for exploiting the antitumor effect of rIL2 without expensive and complicated AIT administration, i.e., by using it in combination with appropriate drugs or BRMs.

A second major area of emphasis in the ETS is to investigate the mechanism(s) by which BRMs augment natural immunity in vivo. Special areas of study include effector cell types, the degree of anatomical compartmentalization associated with BRMs, the in vivo dynamics of leukocyte localization and the possible immunoregulatory participation of NK cells in acute inflammation. Studies are currently under way to determine the ability of BRMs to augment NK-mediated cytotoxicity and to induce antimetastatic effects in nonlymphoid organs, which are often the sites of metastases during the progression of human cancer. In particular, the liver is being used as a model nonlymphoid organ. The results to date have demonstrated that many BRMs, including pyran co-polymer, synthetic polyribonucleotides and rIL2, augment NK activity to a greater degree in the liver than in the blood and spleen. Further studies performed to determine the mechanism(s) by which BRMs augment NK activity and cause LGLs to accumulate in the liver have utilized the bone-seeking isotope strontium-89 to preferentially eliminate BM cellularity. Results suggest that much of the increase in liver-associated NK activity induced by BRMs occurs through an accumulation of NK cells newly derived from BM progenitors.

The ETS has also studied the effect of various BRMs on the migration of leukocytes into the peritoneal cavity in vivo. These studies have demonstrated that IL1 (α and β) and TNF (α and β) are all potent inducers of the acute inflammatory response, as assessed by the increase in polymorphonuclear neutrophils in the peritoneal cavity, with IL1α and IL1β producing the most potent stimuli. TNF (α and β) appears to be 50-100 times less potent. However, some synergy occurs between suboptimal amounts of IL1 and TNFα. Other lymphokines tested at equivalent protein concentrations show no activity; these include IL2, IFNγ, IFNα, IFNβ, granulocyte CSF, granulocyte-macrophage CSF, IL3 and various irrelevant proteins (e.g., human IFNγ, human granulocyte-macrophage CSF and bovine serum albumin). The mechanism of action does not appear to involve prostaglandins or leukotrienes. Since IL1 is only weakly chemotactic in vitro, it is likely that this lymphokine may exert its major effect in vivo on the vascular endothelium.

In addition, the ETS has assessed the effects of lymphokines on the induction of NK activity in the peritoneal cavity. In these studies, IL1 and TNF are without apparent effect on peritoneal NK activity in vivo. In contrast, the injection of high levels of IL2 or lower levels of IFNγ substantially increases peritoneal NK activity. However, IFNγ and TNF have little direct effect on NK activation in vitro. Low levels of IFNγ and IL2 show strong synergy in inducing peritoneal NK activity, and MoAbs to IFNγ can drastically reduce NK levels in response to high-dose IL2 in vivo. These results suggest that IFNγ may play an important role in the augmentation of NK activity by IL2 in vivo. Since IFNγ cannot directly activate NK cells in vitro, we conclude that IFNγ may exert an indirect effect on the vascular endothelium in vivo. This could result in an accumulation of blood-derived NK cells or pre-NK cells in the peritoneal cavity; these cells would then be activated by IL2. Future studies will examine BRM-induced chemotactic factors for LGLs and will attempt to determine whether BRMs induce changes in the binding of LGLs to vascular endothelium.

The ETS also analyzes the potential antitumor activity of selected combinations of BRMs, with the hypothesis that a combination of cytokines will be significantly more effective than either one alone. Particular attention is being given to combinations containing rIL2, rTNF and IFNα for the therapy of murine renal cancer, while IFNγ, IL1 and TGFβ are being used in combination with lipopolysaccharide (LPS) to treat retrovirus-immortalized, neoplastic myelomonocytic cells (GG2EE). Preliminary studies have shown that recombinant murine IFNγ (rmIFNγ) and LPS can partially inhibit the growth of GG2EE in vitro, but rmIFN plus LPS more completely inhibit GG2EE proliferation than either BRM alone. Furthermore, the effect of LPS can be partially duplicated by using rIL1 or rTNF in conjunction with rmIFNγ. Studies are under way to determine whether rIL1, rTNF, TGFβ and rIFNγ can be used therapeutically against GG2EE in vivo. Similarly, since GG2EE expresses the v-_myc_ and v-_raf_ oncogenes as a result of the immortalization of BM cells by the J2 retrovirus, studies are being performed, in collaboration with Drs. Varesio and Young (LMI), to determine whether transient transfection of antisense oligonucleotides will alter expression of v-_myc_ and v-_raf_ and therefore alter the tumorigenicity of the GG2EE cell line.

A fourth major effort of the ETS has been to develop CIT approaches for the intracavitary treatment of human ovarian (OVCAR) and colon (Ht-29) tumors. In a nude mouse xenograft model, intraperitoneal administration of optimal doses of cyclophosphamide and cis-platinum resulted in a remission period of 4 weeks before relapse and death. The concomitant administration of WR-2721, a chemoprotective agent, permitted us to safely deliver much higher doses of cytoxan, with enhanced antitumor efficacy, as determined by a longer remission and increased survival of tumor-bearing mice. In addition, the administration of multiple intraperitoneal treatments of human MoAb conjugated to either the ricin A-chain or _Pseudomonas_ exotoxin has further increased the survival times of mice bearing either OVCAR or Ht-29. These results suggest that the combination of cycle-active chemotherapy with antigen-specific MoAb conjugates can result in an additive or synergistic therapeutic effect.

SUMMARY

Overall, the three sections of the LEI are organized to provide an integrated, complementary approach to the study of the immunomodulatory and immunotherapeutic properties of BRMs. The sections also facilitate the research efforts of other areas of the BRMP by providing expertise in cellular immunology and preclinical animal modeling. The ultimate goal of the LEI is to provide a focus within the BRMP for sufficiently developing immunological approaches and testable hypotheses for cancer treatment so that they can be considered for intramural and extramural clinical trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09228-08 LEI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Further Characterization of Natural Killer (NK) Cells in the Rat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. W. Reynolds	Head	CMIS, LEI, NCI
Others:	D. Reynolds	Biologist	LEI, NCI
	M. Galli	Visiting Fellow	LEI, NCI

COOPERATING UNITS *(if any)*

Pittsburgh Cancer Institute, Pittsburgh, PA (J. Hiserodt); University of Oklahoma (J.A. Murphy); Immunology Branch, Division of Cancer Biology Diagnosis, National Cancer Institute (P. Henkart; W. Munger); Harvard Medical School (R. Stevens).

LAB/BRANCH

Laboratory of Experimental Immunology

SECTION

Cellular and Molecular Immunology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The present studies further characterize natural killer (NK) cells in the rat. The results demonstrate substantial differences in the cells which mediate NK activity (large granular lymphocytes (LGL)) and normal rat T cells. Studies on lymphokine-activated killer (LAK) cells indicate that the major precursor of LAK activity in the rat is an LGL. Analysis of the granules from LGL have led to the isolation of two cytolytic proteins (cytolysin and natural killer cytotoxic factor), at least two BLT-esterases, LGL specific chondroitin sulfate A glycosaminoglycans and proteoglycans, and an anti-fungal molecule which inhibits the growth of Cryptococcus neoformans. These results further characterize the functional nature of NK cells and suggest that LGL represent an entirely separate third lineage of lymphocytes, distinct from both T and B cells.

PROJECT DESCRIPTION

PERSONNEL

Craig Reynolds	Head	CMIS, LEI, NCI
Della Reynolds	Biologist	CMIS, LEI, NCI
Maria C. Galli	Visiting Fellow	CMIS, LEI, NCI

OBJECTIVES

The objectives of this project are: (1) to investigate a number of general biological properties of large granular lymphocytes (LGL), (2) to study the role of LGL in the generation of lymphokine-activated killer (LAK) cells, (3) to further characterize the transplantable spontaneous LGL leukemias in F344 rats, and (4) to examine the mechanism(s) by which LGL mediate their functional activity.

RESULTS

I. Characteristics of LGL

Detailed studies on isolated rat LGL have demonstrated these cells to be a distinct population of cells, about 75 μm2 in size, positive for acid phosphatase and β-glucuronidase; and negative for alkaline phosphatase, esterase, peroxidase and lysozyme. Rat LGL are an antigenically distinct population of cells that share a number of cell surface antigens with monocytes, suppressor/cytotoxic T cells and polymorphonuclear leukocytes (PMN). Unlike T cells, LGL are nonrecirculating lymphocytes with a very rapid precursor turnover time (approximately 10 days). These results are consistent with our observations of LGL present in the red pulp of the spleen but absent from the areas of lymphocyte recirculation; spleen (white pulp), lymph nodes and thoracic duct. Analysis of the interleukin 2 (IL2)-receptor using monoclonal antibodies and Northern blot analysis for mRNA has also demonstrated the absence of a classical 70 kD chain for the IL2-receptor. This result is in spite of the fact that LGL respond to IL2 by 1) increasing their NK activity, 2) growth, 3) activation of LAK activity and 4) production of interferon-γ (IFN-γ) and interleukin 1 (IL1). Further studies on the nature of the IL2-receptor in LGL are currently underway.

II. Role of LGL in the Generation of LAK Cells

In collaboration with Dr. John Hiserodt (Pittsburgh Cancer Institute), these studies demonstrated that LAK activity could be generated from spleen, peripheral blood, lymph nodes, thymus and bone marrow. Spleen cells and peripheral blood lymphocytes respond quickly to recombinant IL2 (rIL2) giving peak LAK activity by 3 to 5 days in culture. Thymus and bone marrow cells were slower to respond peaking by 7 to 9 days in culture. We interpret these data to indicate that thymus and bone marrow contain low levels of LAK progenitor cells which must first expand prior to developing LAK activity. Cells obtained from peritoneal exudate or neonatal spleen cells were unable to generate LAK activity due to active suppressor mechanisms.

Extensive surface marker analyses was performed to determine the phenotype of the LAK precursor and effector cells. LAK precursors expressed surface markers characteristic of NK/LGL since the generation of LAK activity could be significantly reduced by treatment of spleen cells with antibodies to LGL determinants (asialo GM1 or laminin) but not with antibodies to the pan T cell marker, R1-3B3. Furthermore, depletion of granular lymphocytes by the lysosomotropic agent L-LME depleted NK activity and prevented the generation of LAK activity. This same treatment, however, did not affect T cell proliferative responses to Con A.

Preparations of ultra-pure LGL (specifically devoid of T cell contamination by panning with the R1-3B3 MoAb) were fully capable of generating LAK activity. These same populations of ultra-pure LGL demonstrated strong proliferative responses to rIL2 alone. Similar preparations of highly purified resting T cells were unable to generate efficient LAK activity, even when cultivated with an additional activating signal, Con A.

Cell sorting analysis of the LAK effectors demonstrated that LAK effectors clearly sorted into the pan T (R1-3B3) and helper T (CD4) negative populations. The majority (>90%) of the LAK activity sorted into the CD8+, Ia+, and laminin+ populations. These studies indicate LAK cells can be generated from a variety of lymphoid organs in rats and indicate that the major (if not only) cells responding to rIL2 to generate LAK activity are LGL/NK cells.

III. Comparison of Spontaneous Rat LGL Leukemias With Normal Rat LGL

Our recent discovery of spontaneously occurring LGL tumors in aged Fischer (F344) rats has provided a very useful system for obtaining a large number of LGL for detailed biochemical and genetic studies. The rat NK (RNK)-leukemias are morphologically identifiable as LGL with azurophilic granules in the cytoplasm. The RNK lines efficiently kill the NK-sensitive targets (YAC-1, G1-TC), but have little or no activity against the NK-resistant targets [(C58NT)D and P815], and have been used as a convenient source of highly active cells for the isolation and analysis of recognition receptors, cytoplasmic granules, and lytic machinery. We have recently described three new rat LGL tumor lines which can be cultured in vitro without the loss of LGL morphology or function. Two of the lines were initiated in IL2 with the subsequent establishment of both IL2-dependent and IL2-independent sublines. In spite of the IL2 dependence of some lines for growth, no rat IL2 receptor expression was observed using a monoclonal anti-receptor antibody (OX-39) or Northern blot analysis of mRNA using an IL2-receptor specific probe. The morphology of all the cultured LGL tumor lines was consistent with that of LGL; with a kidney-shaped nucleus and prominent azurophilic granules in the cytoplasm. Like normal rat LGL the lines were CD2 (OX-34), CD8 (OX-8), CD45 (T200) and asialo GM1 positive; but negative for the pan-T cell CD5 (R1-3B3, OX-19) marker, CD4 (W3/25) and a variety of other T cell and macrophage associated antigens. Unlike most normal rat LGL, the LGL tumor lines were strongly Ia (OX-6) positive. Two of the three lines killed a variety of NK-sensitive tumor targets but not NK-resistant target cells. All the cultured LGL tumor lines exhibited significant antibody-dependent cellular cytotoxicity (ADCC). In spite of the addition of exogenous IL2 or even the continuous presence of high concentrations of IL2, no LAK activity was observed in any of the in vitro grown lines. All three lines were shown to lack mRNA for either the α or β chains of the T cell receptor. The existence of these lines with high levels of NK activity in the absence of LAK activity now provides a mechanism to study a number of in vitro questions requiring very large numbers of functionally active "pure" LGL in the absence of any contaminating cells.

IV. Analysis of Functional Molecule(s) from the Cytoplasmic Granules of LGL Tumor Lines

The LGL leukemias have previously been shown to be an excellent source of cells for projects which involve the isolation and characterization of subcellular products which may be present in LGL in very small amounts. The use of LGL tumors for these studies is critical since the amount of material required to do these detailed experiments clearly cannot be obtained from normal LGL.

In collaboration with Dr. Pierre Henkart (Z01 CB 05018-18), we have identified at least one molecule from LGL granules which is highly cytolytic. This molecule has not been found in the cytoplasmic granules from other cells; including resting T cells, PMN's, mast cells and macrophages. Antibodies produced in rabbits against the LGL granules were found to be highly inhibitory for both rat and human NK and ADCC activity. Further purification of the cytolytic molecule within the granules suggests that it is a protein of approximately 60,000 MW and acts via polymerization and insertion into the target cell membrane (similar to poly C9 complement pores).

In addition to this cytolytic molecule we have been able to identify at least two BLT-esterase molecules (MW 60 kD and 25 kD) in the granules of LGL. Each esterase can be separately isolated and antisera against each esterase specifically inhibits their respective activity. Studies are currently underway to examine their possible functions in vivo and in vitro.

In collaboration with Dr. Richard Stevens (Harvard Medical School) we have also examined rat LGL for the presence of proteoglycans and glycosaminoglycans in their cytolytic secretory granules. When isolated rat LGL tumor cells were incubated with [35-S]sulfate and the 35-S-labeled macromolecules were purified by density-gradient centrifugation, they eluted on Sepharose CL-4B column predominantly as approximately 500 kD macromolecules. Pulse-chase experiments revealed that these larger macromolecules were proteoglycans that, with time, were processed to glycosaminoglycan-sized macromolecules (approximately 85 kD). As assessed by their susceptibility to chemical and enzymatic degradation and by high-performance liquid chromatography, the LGL-associated proteoglycans bore almost exclusively chondroitin sulfate A glycosaminoglycans. Northern blot analysis, using a gene-specific probe revealed that both normal peripheral blood and transformed rat LGL expressed the same 1.3 kb mRNA that encodes the peptide core of the proteoglycan present in the secretory granules of rat and mouse mast cells. In vivo radiolabeling of rat LGL tumor cells and isolation of their intact granules established that these glycosaminoglycans compartmentalized with the cytolytic activity. These results suggest that the negatively-charged macromolecules may play a role in the regulation, packaging and possible delivery of the cytolysins and basically-charged serine proteases discussed previously.

The granules have been shown to affect the in vitro growth of the fungus Cryptococci neoformans (Dr. June Ann Murphy, University of Oklahoma). Pretreatment of Cryptococci with whole granules or soluble granule contents completely inhibited the growth of this microorganism. Addition of anti-granule Ab or removal of Ca+2/Mg+2 blocked this effect, suggesting that a similar molecule may be affecting the growth of these fungus and causing the lysis of tumor cells. The LGL granules from these tumors have also been shown by Dr. Arnold Greenberg (University of Manitoba) to contain molecule(s) which: 1) will activate the bacteriocidal activity of macrophages and 2) leads to the chemotaxis of monocytes and LGL. Further characterization of the granule associated molecules and a comparison with the antitumor and antifungal activity of LGL granules is underway.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09247-08 LEI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Natural Cell-Mediated Immunity Mechanism of Lysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. R. Ortaldo	Chief	LEI, NCI
Others:	C. W. Reynolds	Head	CMIS, LEI, NCI
	D. Reynolds	Microbiologist	LEI, NCI
	R. Winkler-Pickett	Microbiologist	LEI, NCI
	T. Bino	Visiting Fellow	LEI, NCI
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COOPERATING UNITS (if any)

Immunology Branch, Division of Cancer Biology Diagnosis, National Cancer Institute (P. Henkart); Program Resources, Inc. (J. Rossio).

LAB/BRANCH

Laboratory of Experimental Immunology

SECTION

Cellular and Molecular Immunology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Natural killer (NK) cells and K cells mediating antibody-dependent cellular cytotoxicity have been shown to be large granular lymphocytes (LGL). The majority of LGL form lytic conjugates with a wide variety of NK-susceptible target cells. The relationship of soluble factors to this cell mediated lysis, has been examined using NK cytotoxic factors (NKCF) as the mechanism of action. Three distinct steps have been defined for NKCF: a) production, b) binding to targets, and c) subsequent target lysis. Using rat LGL cell lines as a source for NKCF, we have begun its molecular cloning with the aid of the specific monoclonal antibody to NKCF. Since, the anti-NKCF MAb's were prepared against purified cytolysin, we examined their reactivity against purified granule-derived cytolysin. The data clearly demonstrated an inhibition of lysis by purified cytolysin, but only after Ca++ events. In addition, since nuclear degradation has been implied in cellular lysis, NKCF was examined for this property. NKCF was capable of mediating rapid nuclear degradation of YAC, which was abrogated with the anti-NKCF MAb. In addition to lytic mechanisms, studies are proceeding to define the receptors and structures involved in NK recognition. An anti-idiotypic antibody (anti-ID) has also been developed against a target monoclonal antibody capable of blocking LGL binding, anticipating that this antibody would recognize the NK receptor and aid in its identification. This anti-ID antibody is reactive with a 85 kD protein and blocks LGL binding and target cell lysis. Finally, when LGL were pretreated for 18 hrs with the F(ab')2 anti-ID antibody, there was significant enhancement of lysis and production of IFN-γ. It will be important in future studies to characterize the expression of the 85 kD molecule on activated cells that have reported NK activity. Further biochemical characterization of the NK-R and its cloning are anticipated in future studies.

PROJECT DESCRIPTION

PERSONNEL

John Ortaldo	Chief	LEI, NCI
Craig W. Reynolds	Head	CMIS, LEI, NCI
Della Reynolds	Microbiologist	CMIS, LEI, NCI
Robin Winkler-Pickett	Microbiologist	CMIS, LEI, NCI
Tamar Bino	Visiting Fellow	CMIS, LEI, NCI
Joyce Frey-Vasconcells	IRTA Fellow	CMIS, LEI, NCI

OBJECTIVES

The objectives of this project are:

1. To study natural cell-mediated immunity to tumors in man and analyze the phenotypic, biochemical, and functional characteristics of the cytotoxic cells;

2. To study the nature and mechanism of cytotoxicity by LGL and to attempt to isolate and characterize soluble cytotoxic factors;

3. To study, isolate and characterize the nature of NK effector cell receptors;

4. To study, isolate and characterize the nature of NK target structures.

RESULTS

I. NK Recognition

The nature of the structure(s) on target cells that are recognized by NK cells is presently not well understood. Studies involving indirect methods to examine the specificity of human and mouse NK cells have led to the conclusion that reactivity is quite broad but demonstrates a selective pattern of killing. In studies of NK target antigens on K562 cells, a MoAb was developed which was capable of blocking LGL binding and lysis. These IgM monoclonal antibodies could remove target glycoproteins capable of inhibiting LGL binding to NK susceptible targets. Biochemical studies indicated that the major monoclonal binding was to carbohydrate determinants on the glycoprotein. Therefore, an anti-idiotypic antibody (anti-ID) has also been developed, anticipating that this antibody would recognize the NK receptor and aid in its identification. This anti-ID antibody is reactive with a 85 kD protein and block LGL binding and target cell lysis. Consistent with the idea that this antibody recognizes the NK receptor, F(ab')2 fragments of the anti-ID antibody were found to react only with CD3- LGL among the leukocytes tested. When LGL were pretreated for 18 hrs with the F(ab')2 anti-ID antibody, there was significant enhancement of lysis as compared with normal rabbit serum (NRS) treated control. After pretreatment, levels of IFN-γ production were also increased. Identification of the NK-R will be important to future studies in distinguishing NK activity from activity of other cell types. As seen with fresh, uncultured CD3- LGL, the 80 kD receptor was expressed on IL2 activated CD3+ T lymphocytes and also on a series of CD3+, TcR $\alpha+\beta+$, or $\gamma+\delta+$

expressing clones. In addition, the low level of expression of a single CD3+, TcR $\alpha+\beta+$ clone was unexpected. In addition biochemical examination of the molecule on fresh CD3+ and CD3- lymphocytes determined that a changes in the apparent molecular weight was seen, that may be due to changes in glycosylation. When functional studies were performed on IL2 activated CD3+ and CD3- populations, inhibition was evident however the degree was considerably less than seen with comparable levels of antibody using fresh CD3- lymphocytes and measuring NK activity. However, the functional nature of this receptor was verified by using cross-linked F(ab')2 antibodies and demonstrating specific lysis directed by half of the hetero-antibody. The ability of such cross-linked antibodies to mediate directed lysis has only been shown with functional receptors. However taken together with our lysis inhibition data, we would conclude that other receptor ligand interactions play a major role in IL2 activated killing (LAK), whereas, this NK receptor is of primary importance in fresh killing. The functional significance of this molecule in non-MHC restricted killers like the CD3+, TcR γ/δ cells and its acquisition on CD3+, TcR α/β CTL which demonstrate specific MHC restricted lysis needs further studies. These data suggest that part of the non-MHC restricted lysis mediated by IL2 activated CD3+ and CD3- lymphocytes employ the NK receptor molecule. However, other molecules including the TcR are presumably important in this activated lysis, since the anti-ID is not a potent inhibitor of LAK lysis. Therefore the continued study of the receptors and target structures involved in NK and LAK are warranted, however this reagent and monoclonal antibodies that are being presently developed should improve our ability to study and understand these lytic mechanism(s). The NK-R will be useful in clarifying NK cell lineage, and in the identification of the NK-R ligand. The defined NK-R can be used to identify NK active cells in mixed populations. Present studies are involved in studying the expression of the 85 kD molecule on activated cells that have reported NK activity (CD3+, Leu19+, T-cells, B cells, macrophages, etc.) to determine if these cells express and utilize the same receptor. MAb to the 85 kD molecule are also being produced. Further biochemical characterization of the NK-R, and its cloning are anticipated in future studies.

II. Mechanism of Human NK Activity

Using NKCF as a model for cytolysis by LGL, we have analyzed a variety of agents previously demonstrated to inhibit NK activity. All of the inhibitors will be tested for their effects on: 1) production of NKCF after target-effector interaction; 2) binding of NKCF to target cells; and 3) NKCF lysis (after 6 hrs of adsorption and washing) of targets. In addition, purification studies indicated that radiolabeled NKCF has an apparent non-reduced molecular weight between 20,000 and 40,000. Culture supernatants from rat LGL tumors were shown as an excellent, constitutive source of NKCF activity for NK-susceptible targets, MBL-2 and YAC-1. Mouse monoclonal antibodies were developed which significantly and specifically inhibit the NKCF cytolytic activity of these LGL-derived supernatants. With results indicating that NKCF activity was in fact different from the activities of LT, TNF, complement mediated lysis and leukoregulin, the question of a possible relationship between NKCF and cytolysin needed to be re-addressed. The direct effects were examined by adding anti-NKCF MAbs to granules during the rapid lysis of the NK-sensitive YAC-1 tumor targets. Inhibition could be seen using as little as 25 μg/ml of antibody. The IgM antibody control had little or no effect on lysis. Considering that granules contain a variety of compounds, including cytolysin (BLT-esterase, etc.) (2), it was necessary to examine the ability of anti-NKCF MAbs to neutralize purified cytolysin. The anti-NKCF MAb A0462 significantly inhibits lysis by cytolysin at very low concentrations (<2 μg) of antibody. It should be noted that the anti-NKCF MAb does not bind to cytolysin in the absence of Ca^{++} based on chromatography experiments.

Recognizing differences in the ability of RNK-NKCF to lyse YAC-1 and MBL2, questions were raised regarding whether the mechanism(s) of lysis by RNK-NKCF differs from the mechanism(s) of lysis exhibited by other known cytokines. To begin to examine this question, RNK-NKCF and mrTNF was used to pretreat 3H-Tdr labeled YAC-1 for 0.5, 1 or 2 hours after which time the YAC-1 tumor cells were monitored for the presence of H-Tdr· The results as demonstrated that short exposure (<2hr) to RNK-NKCF results in an increased nuclear degradation potential over that of TNF. With the availability of the anti-NKCF MAbs, the effect of RNK-NKCF on the nucleus of tumor cells could be more directly assessed. For these experiments, MBL-2 and YAC-1 tumor cells were labeled with ^{125}I-deoxyuridine. Labeled cells were treated with RNK-NKCF and antibodies for 18 hours or 4 hours, respectively, after which time nuclear degradation was assessed on an 0.8% agar gel. The data showed that the addition of RNK-NKCF showed DNA degradation of the tumor cells. This pattern is not altered by the addition of the IgM control antibody nor is it altered by the addition of a polyclonal rabbit antisera raised against murine TNF. However, the nuclear degradation pattern by RNK-NKCF is diminished or abrogated by the addition of anti-NKCF MAbs. The use of these MoAbs against NKCF should be very useful in further purification, biochemical characterization and molecular cloning of NKCF and in studying its role in a variety of cell-mediated cytotoxicity assays.

PUBLICATIONS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09256-06 LEI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural Cell-Mediated Immunity: Biology and Regulation of CD3- LGL

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. R. Ortaldo	Chief	LEI, NCI
Others:	A. Mason	Biologist	LEI, NCI
	H. Young	Expert	LMI, NCI
	F. Ruscetti	Head, Lymphokines Section	LMI, NCI
	H. Porter	Bio. Lab Tech.	LEI, NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc., NCI-FRCF (J. Rossio, R. Overton, J. Talmadge; Medicine Branch, Division of Cancer Treatment (Dr. Ozols); Pittsburgh Cancer Institute (R. Herberman)

LAB/BRANCH
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Cellular and Molecular Immunology Section

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NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interleukin 2 (IL2) has demonstrated a potent ability to augment NK activity and the generation of killers against NK insensitive targets. This IL2-mediated augmentation appears to parallel production of IFN-γ by LGL, but abrogation of antiviral activity with anti-IFN-γ serum did not abolish NK boosting. These in vitro results are consistent with the hypothesis that IL2 is the major inducer of LAK activity and that other recombinant cytokines do not appear to regulate either the progenitor or effector stages of LAK activity. In addition to mediating NK activity, LGL have been shown to produce a variety of lymphokines (IL1, IFN, CSF, BCGF). A project investigating gene expression and regulation in highly purified human LGL and T cells is being conducted. Within 1 hr after IL2 treatment of freshly isolated human LGL, IFN-γ mRNA can be detected with IFN-γ protein in the culture medium within 4-6 hours of treatment. These results indicate that with certain stimuli LGL may be the predominant source of IFN-γ from peripheral blood lymphocytes. The methylation state of the T cell receptor β-chain gene (T-β) DNA of T cells, LGL, B cells and monocytes is being studied to determine if it can be used as a marker for different leukocyte populations. In addition to IL2, the interaction of specific surface proteins, as potential regulatory elements are being examined. Specific monoclonal antibodies have been used to examine this question. The results have demonstrated that anti-CD2 monoclonal antibodies have significant regulatory abilities on functions of CD3- LGL. In addition, preliminary results have demonstrated that combinations of antibodies with specificity for selected epitopes of CD2 can result in significant synergy as determined by NK activity and IFNγ production.

PROJECT DESCRIPTION

PERSONNEL

John Ortaldo	Chief	LEI, NCI
Anna Mason	Microbiologist	CMIS, LEI, NCI
Howard Young	Expert	IS, LMI, NCI
Frank Ruscetti	Head	LS, LMI, NCI
Hilda Porter	Bio. Lab. Tech.	CMIS, LEI, NCI

OBJECTIVES

The objectives of this project are:

1. To study the factors regulating the activation and development of natural killer (NK) and related natural effector cells;

2. To analyze the interaction of natural effector cells with other components of the immune system;

3. To study cultures and clones of large granular lymphocytes (LGL) for their cytolytic activity and their production of soluble products in regulation of the immune system.

RESULTS

I. Regulation of Human NK Activity

Studies have been performed to examine the phenotypes of the progenitor and effector cells and to compare these with the cells described as lymphokine activated killer (LAK) cells. Both CD3+ CD16-, and CD3- CD16+ cells could mediate LAK lysis against fresh tumor cells. However, the NK population forms the largest single component of precursor cells for generation of LAK activity in the human peripheral blood. Therefore, these in vitro results are consistent with the hypothesis that IL2 alone induces LAK activity and that other recently described recombinant cytokines do not appear to regulate either the progenitor or effector stages of LAK activity. Because of these similarities, we were interested in examining more extensively the recognition events of fresh NK cells and IL2 activated LAK effector cells. Two approaches were used to examine the recognition events of NK versus LAK. First, the standard cold-target competition method was employed, using unseparated peripheral blood mononuclear cells as well as purified subsets of CD3- LGL and CD3+ T cells as effector cells. When LAK effectors (CD3- LGL, or CD3+ T cells) were tested against chromium-labeled K562 cells, inhibition was seen when K562 cells were used as a cold competitor. Neither FeMX nor ovarian cells nor MBL2 cells (not shown) demonstrated a dose-dependent inhibition of K562 lysis. However, when FeMX cells were employed as targets, FeMX, K562 and fresh ovarian tumor cells competed equally well, with all three effector cells. These results suggest that the LAK recognition structure present on FeMX cells (an NK-insensitive, LAK susceptible target) is significantly different than what is recognized by unactivated NK cells or LAK cells with K562. In order to directly determine if the cells mediating LAK activity to FeMX demonstrate differential recognition and lysis, effectors were separated into FeMX binders and non-binders, then tested against K562 and FeMX. These results clearly demonstrated that the FeMX binders only lysed FeMX, whereas both the FeMX binders and non-binders lysed K562. These data taken collectively with the inhibition and blocking data above indicate a difference in recognition structures involved in NK and LAK.

One of the more interesting findings regarding IL2 activation of human LGL is the multiple activation processes that occur. IL2 activation of LGL increases NK activity, induces IFN-γ secretion, and promotes growth. Initially, our results could not be explained, since $CD3^-$ LGL were IL2 receptor negative, as defined by anti-TAC. Our observations led us to postulate that IL2 could mediate both early and late activation events and that LGL were using a receptor other than the TAC receptor for these cellular events. Although these results were somewhat confusing, they led to the discovery of the β-chain of the IL2 receptor, which has been demonstrated to be constitutively present on fresh, unactivated $CD3^-$ LGL. Further studies are underway to characterize the β-chain of the IL2 receptor in LGL, and its regulation.

II. Non-cytotoxic Functions of Human CD3- LGL

A project investigating gene expression and regulation in highly purified human LGL and T cells is being conducted with Dr. H. Young (Laboratory of Molecular Immunoregulation, BRMP). Within 1 hr after IL2 treatment of freshly isolated human LGL, IFN-γ mRNA can be detected with IFN-γ protein in the culture medium appearing within 4-6 hours of treatment. CD3- CD16+ LGL require only a single signal for IFN-γ production since phytohemagglutinin (PHA), phorbol myristate acetate (PMA), IL2 or ionomycin can each independently induce IFN-γ production. PHA and ionomycin (but not IL2) show significant synergy with PMA as a stimulus to LGL. In contrast, CD3+ T cells require two stimuli for high levels of IFN-γ production and not only are phorbol myristyl acetate (PMA) plus ionomycin or PHA synergistic, but in addition, IL2 and PHA demonstrate some synergy. Furthermore, we have found by fractionation of peripheral blood lymphocytes that IL2 induced IFN-γ production is associated with the LGL population and not T cells. These results indicate that with certain stimuli LGL may be the predominant source of IFN-γ from peripheral blood lymphocytes. Molecular analysis of the T cell receptor genes has shown that functionally active human and rat LGL lack α, β, and γ chain rearrangement or mRNA expression. Therefore, unlike in cytotoxic T cells, the T cell receptor is not involved in the recognition of target cells by LGL. In addition the methylation state of the T cell receptor β-chain gene (T-β) DNA of T cells, LGL, B cells and monocytes was analyzed to determine if methylation can be correlated with the reported transcriptional activity of this gene and to determine if this methylation pattern can be used as a marker for different leukocyte populations. By using the restriction enzyme isoschizomers, (Msp I and Hpa II), we found that T-β of T cells was highly unmethylated, those of B cells and monocytes are highly methylated and the methylation state of T-β of LGL is intermediate between that of T cells and B cells or monocytes. These results indicate that the methylation pattern of T-β can be a marker for T cells and LGL. The difference in methylation pattern of T-β between LGL and T cells and other leukocytes suggested that human peripheral blood LGL are distinct cell subsets. Studies regarding the alteration of DNA methylation and its functional significance are being conducted.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09257-06 LEI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional Activity of Large Granular Lymphocytes in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. W. Reynolds	Head	CMIS, LEI, NCI
Others:	P. Tiberghien	Guest Researcher	LEI, NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

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SECTION

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INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present series of studies have examined the functional activity of rat large granular lymphocytes (LGL), the population of cells known to mediate natural killer (NK) and antibody-dependent cellular cytotoxicity (ADCC). The administration of anti-asialo GM1 antibody decreased NK activity and increased tumor growth, as well as reduced resistance to hematopoietic grafts. The adoptive transfer of LGL into rats with depressed NK/ADCC activity was shown to restore antitumor activity, as well as inhibit the development of syngeneic bone marrow stem cells. These results provide the first direct evidence for an important role for LGL in the control of bone marrow growth and differentiation. These results also suggest that the adoptive transfer of highly enriched LGL populations should be further considered as one potential immunotherapeutic regimen in cancer patients. In addition, protocols involving the depletion of host NK activity in bone marrow transplantation recipients should be further considered. In other experiments we have shown a number of differences in the organ, age and strain distribution between the NK and ADCC effector cell (killer cell) populations. Studies with the BRM, OK432, have shown this agent to augment NK activity and increase survival of tumor-bearing rats.

PROJECT DESCRIPTION

PERSONNEL

Craig W. Reynolds	Head	CMIS, LEI, NCI
Peter Tiberghien	Guest Researcher	CMIS, LEI, NCI

OBJECTIVES

The objectives of this project are: (1) to directly investigate the role of large granular lymphocytes (LGL) in the in vivo resistance to allogeneic and syngeneic bone marrow transplantation and 2) to study the effect(s) of biological response modifiers (BRMs) on NK activity and tumor immunity.

RESULTS

I. The Role of LGL in In Vivo Antitumor and Bone Marrow Transplantation Immunity

Previous studies had shown that rats treated with anti-asialoGM1 (asGM1) antibody showed a parallel decrease in NK activity, frequency of LGL in the spleen and peripheral blood, and clearance of tumor cells from the lungs; indicating that the antiserum-induced depression of NK activity was due to an elimination of most effector cells.

To further determine the possible role of LGL in tumor cell rejection in vivo, we studied LGL involvement in the inhibition of tumor cell metastases in the lungs. Animals treated with anti-asGM1 were found to have a greater than 10-fold increase in the number of lung metastases which developed following the i.v. injection of syngeneic MADB106 mammary tumor cells. Furthermore, the adoptive transfer of 3-10 million highly enriched LGL into NK-depressed animals 2 hours before tumor challenge partially or fully restored the ability of rats to inhibit the development of pulmonary metastases. These results are the first unequivocal evidence that LGL, which are highly enriched in NK activity, play an important in vivo role in the rapid elimination of circulating tumor cells with subsequent inhibition of metastasis.

Similar adoptive transfer studies in anti-asGM1 treated rats have also shown a significant role for NK cells in the inhibition of syngeneic bone marrow stem cell growth and differentiation. Anti-asGM1 treatment of lethally irradiated recipients increased the number of colony forming units in the spleen (CFU-S) following syngeneic bone marrow transfer. The adoptive transfer of LGL but not T cells into these irradiated/anti-asGM1 treated recipients significantly reduced the number of CFU-S enumerated. In addition, in vitro cytotoxicity assays using highly purified LGL and bone marrow stem cells showed significant lysis of the bone marrow cells.

The in vivo administration of anti-asGM1 antiserum to irradiated recipients also reduced resistance to hematopoietic grafts. Using limited doses of T cell depleted bone marrow after lethal irradiation, we have extended previous observations in syngeneic and allogeneic murine combinations and have demonstrated that recipient asGM1 treatment: 1) increases survival in allogeneic as well as in syngeneic BMT combinations; 2) accelerates hematopoietic recovery of peripheral leukocytes, erythrocytes and platelets; 3) increases considerably the amount of donor-derived cells measured at day 7 to day 20 post allo-BMT; 4) does not increase significantly the percentage of donor derived cells measured at day 7 to day 20 post allo-BMT; 4) does not increase significantly the percentage of donor derived cells when evaluated 2 month post allo-BMT. In contrast, when a sub-lethal irradiation (LD80) was given prior to allo-BMT, analysis of chimerism 2 months post BMT demonstrated an increased percentage of donor-derived cells in asGM1-treated recipients. Taken together, these results suggest that recipient NK cells have a potent non-specific inhibitory role on early hematopoietic recovery post BMT. Also, when a suboptimal conditioning regimen is used, NK depletion increases long-term engraftment. These findings should be of value in human BMT and suggest that conditioning aimed at NK-depletion may lead to accelerated engraftment and hematopoietic recovery, increased long-term chimerism and ultimately an increase in survival after BMT.

II. BRM Treatment and Effect on Tumor Growth

The BRM, OK432, was used to assess the effect of BRM treatment on the development of the host's antitumor immune response. F344 rats challenged i.p. with a lethal dose of the syngeneic MADB106 mammary carcinoma could survive more than 100 days when given a single dose of 5 mg/kg of OK-432 i.p. 1 day after tumor challenge. When examining the responsible effector mechanisms in this therapeutic model, two distinct effector phases distinguished by the number of tumor cells were evident. Phase I, 1-6 days following OK-432, resulted in a sharp decrease in tumor cell numbers and was related to the direct anti-tumor cytotoxicity of OK-432 and was coincident with an increase in the number of polymorphonuclear neutrophils (PMN). However, by day 6 a sharp increase in tumor cell numbers was again observed. Subsequently, a second phase of tumor cell destruction was observed 7-20 days following OK-432 and was reflected in a strong lymphocyte-mediated cytotoxicity response as well as the production of complement-dependent cytotoxic antibody against the MADB106 tumor cells. Using an antibody (R1-3B3) and complement depletion of cytotoxic PEC, the MADB106 killer cells appeared to consist of both R1-3B3- (non-T) and R1-3B3+ (T) cells, with most of the anti-MADB106 killing residing in the R1-3B3- cell population. The R1-3B3- killer cells were further defined as: 1) phenotypically asialo GM1+, 2) present in athymic nude rats, and 3) accompanied by some augmentation of YAC-1 killing (the prototype rat NK target), suggesting that some of these R1-3B3- killer cells were typical NK cells. However, it was also observed that most of the R1-3B3- cells which killed MADB106 tumor cells were: 1) phenotypically or functionally different from either cytotoxic T cells or typical NK cells, 2) observed only in MADB106 tumor-bearing rats challenged with OK-432, 3) not present on day 1-2 following OK-432 injection, the time when YAC-1 killing was maximally augmented, and 4) present in high numbers in a secondary response following reinoculation of the MADB106 tumor cells into cured rats. The in vivo relevance and possible derivation of these various cytotoxic lymphocyte populations in the syngeneic tumor-bearing hosts is discussed. In conclusion, this study has demonstrated that the antitumor effects seen with OK-432 are due to a combination of sequential effector mechanisms leading to the eventual rejection of established tumor.

In collaboration with Dr. Robert Wiltrout (Z01 CM 09262-06 LEI) we also studied the augmentation of organ-associated NK activity by a variety of BRMs, including OK-432. The results from these studies demonstrate that BRMs induce a mononuclear cell infiltrate in many lymphoid and nonlymphoid organs. This infiltrate appears to correlate with the rapid appearance of organ-associated NK cells following BRM injection. These results suggest that the rapid infiltration of LGL into sites of inflammation may be an important factor in the subsequent development of mononuclear cell infiltration.

PUBLICATIONS

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Reynolds CW, Sayers TJ. Identification and functional characteristics of lymphocytes with NK activity. In: Jones TC, ed. LSI Monograph on the Pathology of Laboratory Animals, Hemopoietic System. New York: Springer-Verlag, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09303-02 LEI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Drug Resistance in Cancer Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. W. Reynolds	Head	CMIS, LEI, NCI
Others:	W. E. Bere	Biologist	LEI, NCI

COOPERATING UNITS (if any)

Program Resources, Inc., NCI-FCRF (R.R.S. Kantor and S. Giardina); DCT, NCI (K. Cowan and A. Towsen); Regina Cancer Institute, Rome, Italy (P.G. Natali)

LAB/BRANCH

Laboratory of Experimental Immunology

SECTION

Cellular and Molecular Immunology Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.2	0.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present studies examine the mechanisms by which cancer cells develop resistant to cancer chemotherapeutic drugs and seek to devise methods to overcome this resistance. The phase II drug detoxifying enzyme, glutathione S transferase Pi (GST-Pi) has been previously reported to be over-expressed in several drug resistant tumor cell lines. Monoclonal antibodies to GST-Pi have been prepared, characterized and the distribution of GST-Pi determined in normal and neoplastic tissues. GST-Pi appears to be a useful immunohistological marker in cancers of the uterine cervix and in gliomas.

A multimodal approach to cancer treatment has been investigated by the screening of combinations of chemotherapeutic drugs, biologicals and immunotoxins. Human colon carcinoma cell lines have shown greater susceptibility to combinations of 5-fluorouracil and γ-interferon or tumor necrosis factor than any of these agents alone. It is anticipated that these studies will have an important impact on cancer therapy by increasing our understanding of how tumor cells resist therapeutic intervention.

PROJECT DESCRIPTION

PERSONNEL

Craig Reynolds	Head	CMIS, LEI, NCI
William Bere	Biologist	CMIS, LEI, NCI

OBJECTIVES

The objectives of this project are: 1) to study the role of glutathione S transferase Pi (GST-Pi) in malignancy and drug resistance, 2) to utilize information and approaches gained from drug resistance studies in investigation of tumor cell resistance to biologicals and immunological mediators, and 3) to search for ways to overcome the drug resistance of human tumor cells.

RESULTS

I. Monoclonal Antibodies (MAbs)

MAbs have been produced to human GST-Pi isolated from both adriamycin-resistant human breast carcinoma cell lines, ADrR MCF-7 and human placenta. All these MAbs react with both placental GST-Pi and GST-Pi from ADrR MCF-7 cells. These antibodies are isozyme specific as they do not react with cationic or neutral isozymes. MAb AGST 1 and 2 are useful in studying the biochemistry of GST-Pi as they react in Western Blot analysis and immunoprecipitate GST-Pi. Immunohistological studies demonstrate that the reactivity of mAb with GST-Pi is best preserved in heat-fixed cryostat sections of human tissues. GST-Pi is expressed by most human tissues and tumors and appears to be a useful marker in cancers of the uterine cervix and in gliomas.

II. Reversal of Drug Resistance

Human colon carcinomas display an inherent resistance to most cancer chemotherapeutic drugs. A multimodal treatment approach has been undertaken which combines chemotherapeutic drugs, biologicals and immunotoxicins. In preliminary studies, pretreatment of human colon carcinoma cell lines with either γ-interferon or tumor necrosis factor increased susceptibility to 5-fluorouracil mediated cytotoxicity. Additionally lymphokines and cytokines were tested for their ability to increase drug uptake by drug-resistant cells. One lymphokine, leukoregulin, increased adriamycin uptake by two-fold in several tumor cell lines. Presently, combinations of biologicals and immunotoxicins with chemotherapeutic drugs are being evaluated in vitro and promising combinations will be further tested in xenograft mouse model systems.

PUBLICATIONS

Kantor RRS, Giardina SL, Townsend AJ, Longo DL, Natali PG, Myers CE, Cowan KH. Monoclonal antibodies to human glutathione transferase-Pi. Proceedings of the American Association for Cancer Research. New Orleans: American Association for Cancer Research, 1988;29A.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09259-06 LEI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization and Differentiation of NK Cells and Lymphocyte Subsets

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. J. Mathieson	Supervisory Biologist	LEI, NCI
Others:	L. Mason	Microbiologist	LEI, NCI
	R. H. Wiltrout	Head, Exp. Therapeutics Sect.	LEI, NCI
	J. Ortaldo	Chief	LEI, NCI
	A. Wilt	Bio. Lab. Tech.	LEI, NCI
	L. Varesio	Head, Immunobiology Section	LMI, NCI
	H. Young	Supervisory Chemist	LMI, NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc. (S. Giardina, M. Beckwith, C. Moratz, W. Bohn, R. Overton); Memorial Sloan-Kettering Cancer Center, New York City (F.-W. Shen, U. Hammerling).

LAB/BRANCH
Laboratory of Experimental Immunology

SECTION
Leukocyte Differentiation Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies (MoAbs) have been generated or modified to study the origin, differentiation, and potential function of naturally-occurring murine cytotoxic leukocytes. A rat MoAb (4D11) has been generated against a novel, 87 kDa, cell surface antigen on mouse large granular lymphocytes (LGL) with natural killer (NK) activity. This antigen, LGL-1, can be detected on splenic or liver-derived LGL from most strains of mice but not on other types of lymphocytes or hematopoietic populations, and not LGL from other species. Cells electronically sorted for LGL-1 expression are highly enriched for LGL and for NK activity, whereas negatively sorted or MoAb + complement-depleted LGL-1- spleen cells contained little or no NK activity. Dual-color immunofluorescence revealed subsets of LGL-1+ cells which co-expressed Ly-1 (CD5) and Thy-1, whereas CD8+ cells did not co-express LGL-1. LGL-1 can be detected on populations with lymphokine-activated killer (LAK) activity, but it appears that LGL-1 is not expressed on all precursors or effectors of LAK activity. In addition to the 4D11 MoAb, IgG variants of the anti-Qa-5 murine IgM MoAb, recognizing NK cells in some mice, were selected to determine whether IgG MoAbs might be more useful in vivo than the parental IgM. However, the IgG variant MoAbs failed to bind Qa-5 antigen, with sufficient affinity to pursue studies of cellular differentiation and function. A clonal variant producing both IgM and IgG1 was also isolated, which suggested that the sequence for isotype switching may not be parallel to the genomic configuration for the IgH genes. Finally, a MoAb to raf oncogene proteins has been generated and characterized. The antibody, STEGI-1, was generated against purified 30 kDa raf protein and recognizes the immunizing protein and 44-48 kDa protein in a v-raf/v-myc infected murine macrophage line and c-raf or a-raf transfected fibroblasts. Studies on the interaction of oncogene products in normal activation and differentiation of leukocyte subsets and the role of these oncogene products in malignant transformation are now being continued.

PROJECT DESCRIPTION

PERSONNEL

Bonnie J. Mathieson	Supervisory Biologist	LDS, LEI, NCI
Llewellyn Mason	Microbiologist	LDS, LEI, NCI
Robert Wiltrout	Head	ETS, LEI, NCI
John Ortaldo	Chief	LEI, NCI
Alan Wilt	Bio. Lab. Tech.	LDS, LEI, NCI
Luigi Varesio	Head	IS, LEI, NCI
Howard Young	Supervisory Chemist	IS, LEI, NCI

OBJECTIVES

The major objectives of this project are: 1) to identify and characterize the cellular phenotype of leukocytes that have natural killer (NK), or lymphokine-activated killer (LAK) activity, 2) to study the origin, differentiation, development and activation of these cells in vivo and in vitro, 3) to devise new reagents that can potentially target the effects of activated leukocytes at the site of tumors in vivo, and 4) to develop reagents that detect oncogene proteins that might be involved in the activation or functional differentiation of subsets of leukocytes.

Our specific aims are to: (a) acquire and develop a panel of MoAbs and other reagents with selective or differential reactivity against mouse NK cells, (b) use MoAbs to develop enrichment and selection techniques for isolation of precursors and differentiated cells from various tissues, (c) characterize the isolated precursors of augmented NK and LAK cells from spleen, liver, or bone marrow by phenotype, growth requirements in vitro and pattern of cytotoxicity on different tumor cell lines and normal lymphoid targets, (d) detect and analyze the expression of raf oncogene products initially in transfected lines and subsequently in normal leukocyte subsets or cells activated with various stimuli, particularly interleukins and biological response modifiers.

METHODS EMPLOYED

To characterize NK cells and LAK cells in the mouse, we used MoAbs for the mouse that detect: 1) Ly-5, which is expressed on all leukocytes including NK cells; 2) The T cell specific antigens Ly-2 (CD8), L3T4 (CD4) and CD3 as well as Ly-1 (CD5) and Thy-1 which are also expressed on some non-T cells; 3) Qa-5, an MHC-associated, class I antigen expressed preferentially on NK and T cells; 4) NK1.1 asialo GM1 (asGM1) and the Fc receptor for IgG, markers that have been associated with NK cells in mice and other species. MoAbs and other serological reagents for detection of surface antigens were obtained from commercial sources or through collaborative agreements, titered and tested for appropriate reactivity.

I. Subset Enrichment Methods

Nylon wool nonadherent (NW-NA) cells from spleen or liver were subjected to Percoll density gradient fractionation to enrich for low density LGL. The LGL enriched fraction from spleen contains about 10-30% LGL in mice in contrast to the high level of purification seen from peripheral blood cells of humans or rats. Anti-Ig plate adherence was used to further remove contaminating B cells. Anti-Ly-2 (CD8) and anti-L3T4 (CD4) MoAb were also used in indirect anti-Ig coated plate separations and facilitated complement (C)-dependent lysis to further select and/or deplete T cells. Mouse liver interstitial cells that contain a high proportion of LGL were obtained by collagenase digestion of minced perfused livers from MVE-2, Poly IC:LC, or _C. parvum_-treated animals. These cells were further purified by removal of NW adherent cells, Percoll density enrichment, and T cell depletion where necessary.

T lymphocyte subsets and NK cells were purified prior to culture in IL2 for identification and characterization of LAK cells. Purification methods rely on anti-Ig plate adherence of selected specific T subsets treated with MoAb to T cell markers and a final step using selective C-dependent elimination. NK cells expressing LGL-1 were also electronically sorted for analysis of function.

II. Cell Surface Phenotype

Phenotype of the isolated cell populations was determined by one of four methods: a) Flow cytometry analyses (FCA) to detect and quantify cell surface antigen expression detected by immunofluorescence (IF) were performed on an Cytofluorograph and dual-color analyses were performed on a FACScan. b) Cytotoxic elimination with MoAb + C served to directly assess the phenotype of the eliminated cells relative to the level of NK or LAK function, or to eliminate unwanted or irrelevant cell subsets and to enrich LGL before IF or functional analyses. c) For visual assessment of antigen expression on morphologically identified cells, protein A-dependent rosette methods were employed. Finally, d) for indirect assessment of phenotype, absorption of antibody aliquots with various tissue homogenates was done.

III. MoAb Production

Hybridomas producing Ig were obtained by fusion of immunized mouse or rat spleen cells with drug sensitive murine myeloma cells. After selection and expansion in vitro, ascites were produced in nude mice or MoAbs were produced in vitro. Ig from ascites or culture supernatants was purified by sodium sulfate fractionation and column chromatography. Isotype switch variants were obtained by sequential subline selection from decreasing numbers of cells and cloned by limiting dilution.

IV. Antigen Characterization

Molecular characterization of the LGL-1 antigen was performed by immunoprecipitation of surface-labeled (125-I) cell membranes with the 4D11 MoAb. This precipitated material was analyzed on SDS-PAGE gels under reducing and nonreducing conditions. Alternatively, the _raf_ proteins were analyzed by immunoblotting with 125-I protein A for detection of the electrophorectically-separated, detergent-soluble proteins.

RESULTS

I. Enrichment of Murine Cells with NK Activity

Manipulation of lymphoid precursor populations from murine bone marrow or other populations can be informative where relationships between both precursors and functional effectors can be assessed with genetic markers. Therefore, we have continued to develop methodology to identify, isolate, monitor, and assess cells with potential natural cytolytic capacity. We have also continued comparative phenotypic and functional analysis of NK cells, LAK cells, and T cells.

A stepwise selection procedure to isolate different murine lymphocyte subsets from mouse spleen and liver has been devised. This procedure involves depletion of B cells and macrophages by nylon wool, followed by selective adherence of MoAb coated T cells to anti-Ig coated Petri dishes. Finally, the non-T, non-B cells are further depleted by MoAb + C lysis to enrich for LGL/NK cells. The separated populations were then compared phenotypically and functionally before and after culture in IL2 to determine what relationship exists between T cells, NK cells and LAK cells. These data indicated that the non-T cell population enriched for asGM1-bearing cells, contains virtually all of the NK activity before culture and is responsible for nearly all of the LAK activity after IL2 culture for 3-5 days. In contrast, CD8+ T cells which can proliferate in the IL2 are only marginally effective in producing LAK activity.

II. Characterization of NK Cells Using MoAbs

Several MoAbs were generated to study the origin, differentiation, and potential function of naturally-occurring cytotoxic lymphocytes. By blocking the response to common lymphocyte antigens, rat MoAbs were raised to mouse LGL. In particular, one rat MoAb (4D11) was generated that recognizes a non-allelic determinant on NK-active cell populations. The 4D11 antibody recognizes a novel cell surface antigen, and expression is highly correlated with the presence of LGL in B6 mice and related congenics. IF studies indicate that this antigen is expressed on LGL of most strains of mice but it is not detected on thymocytes, T or B lymphocytes or other hematopoietic populations. Antibody-dependent, complement-mediated lysis depletes 50 to 80% of the NK activity in leukocyte populations enriched for NK cells. Cells positively selected by electronic sorting of the 4D11+ leukocytes, contain 50 to 60% LGL and are enriched 7 to 70 fold in their lytic activity against YAC target cells. Preliminary studies indicate that the antigen recognized by 4D11 (LGL-1) is not expressed on all precursors and effectors of LAK or augmented NK activity, nor on thymocytes.

Qa-5 is an antigen present on mouse T cells and all NK cells and is defined by a murine IgM MoAb. Although this MoAb + C completely abrogates NK activity in vitro, when we attempted to use anti-Qa-5 reagent in vivo only a limited anti-NK effect was detected, possibly due to a rapid clearance of the IgM antibody from circulation. To address this problem, we used the process of sequential sublining to select and clone anti-Qa-5 hybridoma cells which have spontaneously switched to production of IgG isotypes. We successfully isolated switch variants of the IgG2a, IgG2b, and IgG1 subclasses. In addition, a separate clone that secretes both IgG1 and IgM simultaneously was isolated. This type of double producing variant suggests that the sequence of plasma cell differentiation for different isotype expression is not collinear with the Ig gene organization, and that the deletion of intervening IgH sequences may not be required for switching. We also determined that the pretreatment of the parent IgM secreting clone with a murine erythroleukemia differentiating agent, hexamethylene-bis-acetamide (HMBA), enhances the number of detectable switch variants approximately 3 fold. However when tested for anti-Qa-5 specificity by binding to C57BL/6 lymph node cells, none of the switch variants maintained high affinity binding to Qa-5+ cells. The IgG2 isotype switch variant was able to competitively-inhibit binding of the parent IgM when used in 50 fold excess. In addition, anti-Qa-5 IgM lost its ability to bind when reduced to monomeric subunits. Therefore, we conclude that although the frequency of isotype variants can be enhanced by HMBA, the affinity associated with a single binding site does not appear to change. Therefore the loss of antigen-specific binding by the isotype switch variants is most likely due to a decrease in affinity when the IgM pentameric structure is lost.

III. Characterization of Oncogene MoAbs and Expression in Activated or Malignant Cells

To elucidate the role of oncogenes in cellular differentiation and malignant transformation, murine MoAb were generated to the *raf* oncogene products. The anti-*raf* MoAb, STEGI-1 was produced against a 30 kDa v-*raf* protein produced in *E. coli*. STEGI-1 is capable of immunoprecipitating the 30 kDa protein used for immunization as well as a 44 kD c-*raf* protein from cells transfected with multiple copies of the *raf* gene or in the *myc*/*raf* transfected cell line GG2EE. Furthermore, application of the lysolecithin technique to permeabilize cells has enabled us to detect *raf* protein in the GG2EE cell line transformed by a *myc*/*raf* viral construct. Thus, cellular expression levels in activated cells may be monitored at the protein level by this technique and we are now applying this approach to study oncogene levels in different subsets of leukocytes.

PUBLICATIONS

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Mason L, Giardina SL, Hecht T, Ortaldo J, Mathieson B. LGL-1: A non-polymorphic antigen expressed on a major population of mouse natural killer (NK) cells, J Immunol 1988;140:4403-4412.

Mason L, Giardina SL, Hecht T, Ortaldo JR, Mathieson BJ. LGL-1: Non-polymorphic antigen expressed on a major population of mouse natural killer cells. In: Lotzova E, Herberman RB, eds. Proceedings of the 5th International Workshop on Natural Killer Cells. Hilton Head: 5th International Workshop on Natural Killer Cells, 1988;in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09282-04 LEI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Potential Differentiation Capacity of Thymocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. J. Mathieson	Supervisory Biologist	LEI, NCI
Other:	T. Gregorio	Bio. Lab. Tech.	LEI, NCI
	L. Mason	Microbiologist	LEI, NCI
	J. Wine	Bio. Lab. Tech.	LEI, NCI
	L. Takacs	Visiting Fellow	LMI, NCI
	F. W. Ruscetti	Head	LS, LMI, NCI
	H. A. Young	Supervisory Chemist	LMI, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc. (K. McConville-Komschlies, R. Overton, L. Finch); LMO, DCE, NCI (N. Bhat and T. Papas); University of Rome "LaSapienza" (I. Screpanti); Arthritis and Rheumatism Branch, NIAMSD, (W. C. Gause); Memorial Sloan-Kettering Cancer Center (F.-W. Shen).

LAB/BRANCH

Laboratory of Experimental Immunology

SECTION

Leukocyte Differentiation Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.0	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The differentiation of murine thymocyte subsets has been further examined. Intravenous (i.v.) and intrathymic (i.t.) cell transfers into irradiated congenic mice, previously had demonstrated a precursor subset of dull Ly-1+ (dLy1) thymocytes lacking CD4 and CD8, i.e. double negative (DN). These dLy1 cells contain committed thymocyte progenitors with limited capacity for generation of other hematopoietic cells. Additional, more immature thymocyte precursors have been found in bone marrow by i.v. and i.t. transfer, but i.t. transfers bypass the need for "homing" of cells to the thymus. This i.t. methodology has enabled us to transfer the CD4+,CD8+, double positive (DP) blasts, and thymocytes expressing only CD4+ or CD8+. The DP blasts differentiate almost exclusively to CD4+ cells, whereas dLy1 cells first yield DP cells, followed by CD4+ and then CD8+ cells. Isolated CD4+ or CD8+ cells are incapable of extensive growth or repopulation of other subsets and isolated NK cells do not become thymocytes. We have also determined that limited pretreatment with estradiol, without irradiation, renders the recipient's thymus transiently receptive for thymocyte precursors. Exogenous estradiol administration selectively depletes a subcapsular thymic blast population, which permits limited thymic repopulation by donor-derived dLy1 or bone marrow cells. However, studies of bone marrow and thymus precursors, examined at multiple time points in standard irradiation chimeras, indicate that although there are marked differences between these two types of precursors, there is no definitive subcapsular localization. The phenotype of rat and mouse DN thymocytes has been compared. The majority of both rat and mouse DN cells are proliferating when first isolated and have low CD5 (Ly-1) and high Thy-1 expression. However in remarkable contrast to the mouse, the rat DN thymocytes reveal no interleukin 2 receptor expression. Finally, continuing studies examining oncogene expression in normal mouse lymphocyte subsets has revealed a selective increase in ets oncogene expression in CD4+ thymocytes compared to other thymic subsets or CD4+ lymph node T cells.

PROJECT DESCRIPTION

PERSONNEL

Bonnie J. Mathieson	Supervisory Biologist	LDS, LEI, NCI
Theresa Gregorio	Bio. Lab. Tech.	LDS, LEI, NCI
Llewellyn Mason	Microbiologist	LDS, LEI, NCI
John Wine	Bio. Lab. Tech.	CMIS, LEI, NCI
Laszlo Takacs	Visiting Fellow	LMI, NCI
Frances W. Ruscetti	Head	LS, LMI, NCI
Howard A. Young	Supervisory Chemist	IS, LMI, NCI

OBJECTIVES

The major objectives of this project are: 1) To study the normal intrathymic differentiation pathways in mice and rats, and to specifically compare the phenotype and functional potential of different thymic subsets by intravenous (i.v.) or intrathymic (i.t.) routes. 2) To determine the differentiation potential of thymocyte precursors that develop in mice pretreated with estradiol or irradiation. 3) To develop enrichment and selection methods to obtain both immature and mature thymic subsets from fetal and adult organs for manipulation in vitro with biological response modifiers (BRM). 4) To develop methods to enhance the transfer and regeneration of thymus-derived cells in irradiated or immune deficient hosts. 5) To compare the transfer of thymic precursors with cells of similar phenotype from peripheral lymphoid organs. 6) To determine the anatomical location(s) of thymocyte progenitors and their descendants as differentiated populations developed. 7) To compare early thymocyte precursors with NK precursors and isolated NK cells. 8) To determine whether immature thymic cells can be shifted toward NK cell differentiation.

METHODS EMPLOYED

Cell suspensions of immature and mature thymocytes isolated by various methods have been used as potential sources for i.v. and i.t. transfers. Immature dLyl, DN adult thymocytes were obtained by lysis of the CD8+ cells with anti-Ly-2.2 monoclonal antibody (MoAb) plus complement (C) treatment, followed by selective cytotoxic elimination of the remaining mature CD4+ subset with anti-L3T4 and anti-Lyl plus C. This complete procedure yields less than 1% of normal adult thymocytes and extreme care is exercised to avoid contamination with blood and peripheral lymphoid tissue. Mature subsets of either CD8+ or CD4+ cells were obtained by first eliminating the reciprocal population by two cycles of MoAb + C treatment then selecting the CD8+ or CD4+ cells by adherence to anti-Ig coated plates. Double positive (DP) blasts were selected by anti-Ly-2 adherence followed by anti-L3T4 adherence before or after enrichment on Percoll density gradients. Rat DN thymocytes were prepared by adherent depletion with reagents appropriate for the rat CD4 and CD8 antigens.

Purification of cell subsets and differentiation in vivo or in vitro was monitored by flow cytometry analysis (FCA) in the BRMP Clinical Monitoring Unit, PRI or on the LEI FACScan. MoAbs and appropriate fluorescent detection reagents have been obtained through the laboratories that produced them, through the ATCC, or through commercial sources for use in these studies.

Congenic animals used as recipients for thymocyte subsets were irradiated (700-1000 rads) or estradiol-treated (50 μg/day x 3 days). These animals have been monitored from 6-40 days for donor and host phenotype after i.v. or i.t. cell transfers. For localization studies, sections of frozen tissue were incubated sequentially with appropriate MoAbs, peroxidase-linked anti-Ig reagents for deposition of peroxidase product.

RESULTS

I. Characterization of Immature Thymocytes

A small subset of mouse thymocytes with a phenotype which suggests that these cells are the equivalent of the thymic subcapsular cells previously has been reported. These cells are dull for Ly-1 (dLy1) by quantitative immunofluorescence, express no detectable CD4 or CD8 (i.e. double negative, DN) and many are large blast cells. These cells have limited self renewal characteristics and are committed to the T cell lineage as demonstrated by: a) their ability to repopulate all subsets of the thymocytes and b) their failure to yield multi-potential colony forming units in the spleen when transferred in vivo to irradiated animals. In addition the expression of IL2 receptor (IL2R) is seen on about 60% of these cells. Finally dLy1 cells appear to be similar in phenotype to early (day 13-15) fetal thymocytes.

Rats, like mice, express CD4 and/or CD8 on most thymocytes and T cells. The CD4-,CD8-, DN subset of rat thymocytes was isolated and the expression of other phenotypic markers was more extensively studied. Like the majority of mouse DN thymocytes, the rat DN thymocyte subset expresses low levels of CD5 and many of the cells are large, low density, blast cells. However IL2R expression cannot be detected on the rat DN subset either by antibodies to the 55 kDa chain or by radiolabelled IL2 binding or by immunoprecipitation after chemical cross-linking. However, the rat thymus does contain some IL2R+ cells, which appear to be restricted to the medulla, but our data indicate that the medullary IL2R+ population is not isolated in the DN population. This medullary localization of IL2R+ cells is also seen in the human thymus, whereas the mouse thymus has both cortical/subcapsular and medullary IL2R+ cells.

Cell suspensions of various thymic subsets or bone marrow populations have been injected into the thymus of irradiated and estradiol-treated mice. These experiments have specifically allowed us to manipulate and assess the differentiation capacity of subpopulations whose potential for homing to the thymus is affected or limited by previous technical manipulations or the differentiation state of the cells. The dLy1 subset can be injected i.t. into irradiated hosts to repopulate the four major subsets within the thymus, and the sequence in which they appear is the same as that previously reported for i.v. transfers of dLy1 cells. In addition, the CD4+,CD8+/DP blast population obtained from the adult thymus, as well as the 16-day fetal thymus, can be shown to give rise to mature CD4+ cells after i.t. injection. Furthermore, donor-derived cells from both the mature CD4+ and CD8+ subsets could be detected after i.t. injection in irradiated hosts. Not all immature thymocytes can be transferred i.t., because attempts to transfer early (day 13-15) fetal thymocytes have been largely unsuccessful, requiring cell numbers at least one log higher than the adult dLyl cells. In contrast, both the DP and the dLyl populations present at 16 days can yield donor-derived cells in irradiated animals. This indicates that the precursor capable of CD4+ or CD8+ subset differentiation is very low in the fetal thymus.

II. Attempts to Transfer Large Granular Lymphocytes (LGL) i.t.

There are both morphological and phenotypic similarities between fetal thymocytes, dLyl adult thymocyte precursors and LGL isolated from mouse spleen or liver. Therefore, we have also attempted to transfer LGL by i.t. injection to determine whether these cells had any potential as thymic precursors. In these studies, we have compared LGL isolated from mouse livers (see accompanying report Z01 CM 09259-06 LEI) with dLyl cells and unfractionated bone marrow which contains the precursors for both NK cells and thymocytes. In these studies, it is evident that i.t. transferred dLyl and bone marrow cells successfully yield donor-derived cells but LGL do not. By contrast LGL or bone marrow injected i.v. can generate donor-derived LGL with NK activity.

III. A Selective Depletion in the dLyl Population by Estrogen-treatment of Animals

We have also examined the ability of different thymic populations to be transferred to congenic hosts treated with estrogen. Because estrogens administered to mice during prenatal and neonatal periods evoked impairment of thymus-derived immune functions, it was postulated that the estrogen receptors on low density thymocytes, which might include dLyl cells, would delete endogenous precursors and permit the transfer of donor-derived cells to specific niches within the thymus. We have tested this hypothesis and have determined that dLyl cells can indeed repopulate the thymus of estrogen-treated animals in a limited fashion. Whereas irradiation has non-selective lympholytic effects, estrogen appears to deplete a selected population of subcapsular thymic cells in the adult animal. Surprisingly, the early donor-derived repopulation in estrogen-treated animals injected with dLyl cells is nearly equivalent numerically to the donor-derived repopulation seen in irradiated animals. However, there is a difference in the proportion of repopulating cells because irradiated animals are more highly depleted at the time of irradiation than are estrogen-treated animals. At least both DP thymocytes and CD4+ mature thymocytes can be generated after i.t. transfer to estrogen-treated congenic animals. We are currently attempting to characterize the population of dLyl DN cells that remains after estrogen treatment.

IV. Localization of Injected Cells and Their Progeny

Studies to monitor the thymic localization of donor-origin thymocytes with time following i.t. or i.v. transfer of thymocyte or bone marrow subsets have been performed using the Ly-5 allelic difference and immunoperoxidase detection of MoAb binding. Donor-derived thymic cells from either i.t. or i.v. injected precursors can be detected from days 6 to 26 for dLyl transferred cells and from days 12 to 40 after i.t. bone marrow transfer. Whereas, i.v. bone marrow permits the establishment of permanent chimeras. In contrast to an expected subcapsular localization, precursors were first observed in the medulla in bone marrow transfers and scattered throughout the irradiated (day 6) thymus of dLyl recipients. The subsequent patterns that were observed suggested that the dLyl differentiation was shifted about 10 days earlier than bone marrow precursors.

V. Studies of Oncogene Expression in Normal and Activated Subsets

In collaboration with investigators in Program Resources, BRI-Basic Research Program, and the LMO, DCE, NCI at the FCRF, we have been analyzed different subsets of thymic lymphocytes and T cells for oncogenes which appear to have selective expression in lymphocytes. Recent studies indicate that ets-oncogenes, particularly ets-1, are selectively expressed in differentiating CD4+ thymocytes, compared to CD8+ or dLyl thymic subsets. Furthermore the CD4+ lymph node T cells no longer show this differential expression of ets oncogenes relative to CD8+ T cells. Earlier studies with c-rel demonstrated that c-rel (the cellular homolog of v-rel, the transforming gene of the leukemogenic reticuloendotheliosis retrovirus) plays a role in later stages of lymphocyte differentiation than has been reported for genes like c-myc or c-ets.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09262-06 LEI

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antitumor Effects of rIL2-Stimulated Lymphocytes, NK Cells & Macrophages in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. H. Wiltrout	Head, Exp. Therapeutics Section	LEI, NCI
Others:	A. M. Pilaro	IRTA Fellow	LEI, NCI
	R. L. Hornung	Biotechnology Fellow	LEI, NCI
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	P. L. Urias	Chemist	LEI, NCI
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COOPERATING UNITS *(if any)*

Preclinical Evaluations Laboratory and Clinical Immunology Services, Program Resources, Inc., NCI-FCRF, Frederick, Maryland 21701; Laboratory of Molecular Immunoregulation, Developmental Therapeutics Program, NCI; Pittsburgh Cancer Institute, Pittsburgh, PA.

LAB/BRANCH

Laboratory of Experimental Immunology

SECTION

Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies from our laboratory have shown that administration of adoptive chemoimmunotherapy (ACIT) consisting of doxorubicin hydrochloride (DOX) and lymphokine activated killer (LAK) cells plus IL2 cured 60-80% of mice bearing established murine renal cancer (Renca). Further studies have shown that LAK cells can be fractionated on Percoll density gradients such that >85% of the total cytolytic activity can be enriched in approximately 20% of the cells in fraction 2 (F2). These F2 cells, when labelled with 111-Indium, do not distribute widely or localize to a significant extent in tumor tissue following intravenous administration, suggesting that the mechanism of in vivo antitumor effects by LAK cells may be largely indirect. Additional studies have shown that the investigational drug Flavone acetic acid (FAA) augments NK activity in normal and tumor-bearing mice and synergizes with rIL2 for the treatment of Renca. FAA + rIL2 is an effective treatment for minimal residual disease, intrarenal primary tumors, and for experimentally established pulmonary metastases. The mechanism by which FAA synergizes with rIL2 involves the induction of IFNα by FAA, requires both asialo-GM1+ and T lymphocytes. Additional experiments involving the potential role of cytokines in cancer treatment have utilized the retrovirus induced GG2EE myelomonocytic tumor cell model shown to be partially growth inhibited by IFNγ, IL1, TNFα, and TGFβ. Very low doses of these cytokines exhibit synergistic antiproliferative effects in vitro. Studies have also been performed which demonstrate that large granular lymphocytes (LGL) can respond chemotactically to activators of protein kinase C. These results suggest that BRM may induce the localization of LGL in vivo partially through the induction of chemotactic factors.

PROJECT DESCRIPTION

PERSONNEL

Robert H. Wiltrout	Head	ETS, LEI, NCI
Anne M. Pilaro	IRTA Fellow	ETS, LEI, NCI
Ronald L. Hornung	Biotechnology Fellow	ETS, LEI, NCI
Emira Ayroldi	Visiting Fellow	ETS, LEI, NCI
Hoo S. Goey	Guest Researcher	ETS, LEI, NCI
Hitoyasu Futami	Nakasone Fellow	ETS, LEI, NCI
Patricia L. Urias	Chemist	ETS, LEI, NCI
Eilene Gruys	Biologist	ETS, LEI, NCI

OBJECTIVES

The overall objective of this project is to develop preclinical strategies by which chemotherapeutic drugs and recombinant cytokines can be used in concert for the treatment of cancer in mice. Additional studies designed to determine the mechanism(s) by which biological response modifiers (BRM) regulate the localization of natural killer (NK) cells in vivo. Specific objectives are as follows:

1. To determine the mechanism by which the investigational agent Flavone acetic acid (FAA) and rIL2 synergize for the treatment of established murine renal cancer (Renca).

2. To determine the ability of TNFα, IFNγ, TGFβ, and IL1 to synergize for the treatment of Renca and a retrovirus-immortalized mouse myelomonocytic tumor.

3. To determine the mechanism(s) by which adoptively transferred lymphokine-activated killer (LAK) cells plus rIL2 mediate antitumor effects.

4. To study the induction and regulation of large granular lymphocytes (LGL) chemotactic responses by BRM.

RESULTS

There are three major areas of study in progress. First, a major focus of the research is on the development and utilization of combination approaches to cancer treatment in mice whereby chemotherapeutic drugs and cytokines are used in the absence of adoptive immunotherapy. Specific approaches developed include the use of FAA plus rIL2 for the treatment of Renca and the treatment of oncogene-bearing, retrovirus-immortalized GG2EE tumor cells by cytokine combinations. Second, studies have been performed to determine how adoptively transferred LAK cells mediate antitumor effects in vivo. Third, since IL2-activated LAK cells exhibit migration patterns similar to those of NK cells, studies have been initiated to a) determine whether NK cells and LAK cells respond to chemotactic stimuli and b) study the regulatory mechanisms of the chemotactic response by these cell types.

Because NK cells constitute the major precursor population for LAK activity, we have investigated the ability of the investigational agent FAA to modulate NK activity and studied its ability to mediate antitumor effects when used in concert with rIL2. These studies demonstrated FAA to be a potent augmenter of systemic NK activity in normal and tumor-bearing mice at doses $\geq$ 125 mg/kg. Further studies demonstrated that FAA and rIL2 function synergistically in the treatment of murine renal cancer such that 50-80% of Renca-bearing mice could be rendered disease free by this regimen while no mice were cured by FAA or rIL2 alone. Most interestingly this therapeutic activity was achieved without the requirement for adoptively transferred LAK cells and could be performed using doses of FAA (150 mg/kg) and rIL2 (1000 U to 30,000 U/mouse/day) which were not overly toxic. The FAA + rIL2 combination was effective against minimal residual disease following nephrectomy of the tumor-bearing kidney (approximately 60% of all mice were cured of their disease). Similar results were achieved against established intrarenal primary tumors and pulmonary metastases. Depletion of asialo GM1+ cells, presumably NK cells, significantly reduced the effectiveness of the FAA + rIL2 approach. Further, a significant proportion of mice cured of Renca by FAA + rIL2 were immune to rechallenge by Renca. These results suggested that NK cells and T lymphocytes participated in the antitumor effects mediated by FAA + rIL2. FAA has also been shown to induce up to 10,000 U/ml of IFNα in the serum within several hours of administration. However, the administration of high doses of IFNα A/D Bgl, or the use of the IFN-inducer poly ICLC in concert with rIL2, was unable to stimulate the same therapeutic effects induced by FAA + rIL2, suggesting that FAA may induce other cytokines which synergize with IFNα and rIL2.

A similar approach has been used to study the inhibition of tumor cell growth of the GG2EE tumor line in vitro. The GG2EE tumor line was obtained by immortalization of C3H/HeJ bone marrow cells by the J2 recombinant retrovirus which bears the v-myc and v-raf oncogenes in the laboratory of Dr. Luigi Varesio, LMI, BRMP. This cell line offers several advantages for the study of therapy by cytokine combinations. First, the GG2EE expresses both the v-myc and v-raf oncogenes. Therefore, antiproliferative effects of cytokines can be studied in the context of changes in oncogene expression. Second, GG2EE is tumorigenic in vivo in athymic mice and therefore cytokines which exhibit antiproliferative effects in vitro can be studied for efficacy in vivo. Our studies have shown that IFNγ and LPS synergize for growth inhibition of GG2EE in vitro. Synergistic or additive antiproliferative effects are also observed when rIL1 is used in combination with IFNγ and TNFα, while TGFβ also mediates strong antiproliferative effects against GG2EE in vitro. Interestingly, GG2EE can be induced to synthesize rIL1, TNFα, and TGFβ, suggesting that the growth of this cell line can be inhibited in an autocrine manner following the proper stimulation. Experiments are in progress to relate the antiproliferative effects of these cytokines to expression of the v-myc and v-raf oncogenes.

Adoptively transferred LAK cells plus IL2, alone and in combination with chemotherapeutic drugs, have been shown to mediate antitumor effects against a variety of murine tumors. However, the mechanism(s) by which these effects occur remain unclear. We have performed studies to determine whether radiolabelled LAK effector cells can localize in tumors following intravenous transfer. These studies demonstrated that LAK effector cells, i.e. those lymphocytes which mediate cytolysis of tumor cells in vitro, can be obtained in highly enriched form from Fraction 2 (F2) of Percoll density gradients. These 111-Indium-labelled F2 LAK effector cells are rapidly arrested in the lungs, with most of the radioactivity subsequently transferred to the liver. Relatively little radioactivity was detected in the spleen or lymph nodes. In contrast, the small noncytolytic lymphocytes, largely T cells, from F4 migrated efficiently to lymphoid tissue. Neither F2 nor F4 cells exhibited enhanced localization in tumor-bearing vs normal kidneys, suggesting that direct recognition and cytolysis of tumor cells by LAK effector cells is probably not the primary mechanism for LAK cell-mediated antitumor effects in vivo. Studies are in progress to determine which immunoregulatory cytokines are produced by F2 LAK effector cells.

Since LAK cells and NK cells exhibit relatively restricted localization patterns in untreated mice, but NK cells can be induced to accumulate in some organs following BRM treatment, studies were performed to determine whether NK cells can respond to chemotactic stimuli induced by BRM. Initial studies were designed to develop a model for LGL chemotaxis and to identify potent chemoattractants which could serve as positive controls for these studies. We have developed a modified Boyden chamber method for assessing chemotaxis of purified rat LGL whereby LGL can be visualized, immunohistochemically stained, and enumerated upon completion of the assay. A variety of agents have been shown to be chemotactic for LGL, including F-met-leu-phe, complement fragments (C5a), and leukotriene B4. However the best chemoattractants found to date are the tumor promoting phorbol ester TPA, and diacylglycerol. Both of these agents activate protein kinase C (PKC) and inhibition of PKC with the antagonist H-7 significantly impairs the chemotactic response. Thus these studies have demonstrated that LGL can chemotax toward a variety of stimuli and suggest that their response is at least partially dependent on activation of PKC. Overall, these studies should provide insight into how BRM-induced redistribution of leukocytes is regulated and contribute to our understanding of the mechanisms by which BRMs induce antimetastatic effects in nonlymphoid organs.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 09288-03 LEI

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemo-Immunotoxin Therapy Against Human Tumors Xenografted in Athymic Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Pearson	Microbiologist	LEI, NCI
Others:	R. H. Wiltrout	Head, Exp. Therap. Section	LEI, NCI
	D. L. Longo	Associate Director	BRMP, NCI
	J. R. Ortaldo	Chief	LEI, NCI
	D. Fitzgerald	Senior Investigator	LMB, NCI

COOPERATING UNITS *(if any)*
Program Resources Inc. (T. Sayers); XOMA Corp. (P.W. Trown); Cetus Corp. (E. Groves); NeoRx Corp. (C. Morgan)

LAB/BRANCH
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SECTION
Experimental Therapeutics Section

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	1.00	1.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major focus of this project is to explore the therapeutic efficacy of specific immunotoxins (IT) used either alone or in conjunction with chemotherapy via an intracavitary approach against tumors confined to the peritoneal cavities of athymic mice. Two models under investigation are a human colon (Ht-29) and ovarian (OVCAR-3) carcinoma xenografted intraperitoneally (ip) in nude mice. Studies have revealed that following ip inoculation of either Ht-29 or OVCAR-3 a localized disease is presented which is characterized by malignant ascites and solid tumor throughout the peritoneal cavities of mice. Drug studies involving Ht-29 revealed that the administration of 300 mg/kg of cytoxan in combination with 100 mg/kg of WR-2721, at 10 and 17 days, post-tumor cell inoculation resulted in a significant increase (P=.002) in MST (61 days) as compared to a MST of 35 days in the controls. Likewise, the ip injection of 200 mg/kg of cytoxan and 5 mg/kg of cis-platinum to mice bearing OVCAR-3 disease resulted in an increase in survival time, with a range of death 90 to 120 days as compared to a death range of 40 to 50 days in the control group. Mice that received either three or six injections of the IT, OVB-3 (PE), .5 μg, every other day beginning 3 days post-tumor inoculation (3 million), exhibited increased MSTs (P=.002) of 62 and 68 days, respectively, as compared to a MST of 33 days for the controls. OVB-3 alone was ineffective. Nr-Lu-10 (PE) given ip at a similar dose and regimen, to mice bearing the colonic disease showed similar MST's of 50 and 60 days, respectively, (P=.002). Groups of mice that received two, four or seven treatments of OVB-3 (PE) following cytoxan therapy, at a time of minimal tumor burden, exhibited a further significant increase (P=<.002) in MST's to 81, 89, and 96 days, respectively, compared to a MST of 59 in the drug treated group. Similarly, multiple treatments of Nr-Lu-10 (PE) following debulking therapy gave MSTs of 89, 97, and 105 days, respectively (P=<.002). Finally, OVB-3 (PE) is being tested against OVCAR-3 following cytoxan and cis-platinum therapy.

PROJECT DESCRIPTION

PERSONNEL

John W. Pearson	Microbiologist	ETS, LEI, NCI
Robert H. Wiltrout	Head	ETS, LEI, NCI
Dan L. Longo	Associate Director	BRMP, NCI
John R. Ortaldo	Chief	LEI, NCI
David Fitzgerald	Senior Investigator	LMB, NCI

OBJECTIVES

The aim of this project is to evaluate the intracavitary administration of immunotoxins (IT) alone or in conjunction with cytoreductive agents against tumors confined to the peritoneal cavities of athymic mice. The specific objectives of this project are as follows: (1) to develop appropriate models for testing the therapeutic efficacy of ITs in vivo, (2) to define optimal treatment protocols, i.e. dose and regimen to maximize the therapeutic effect of specific ITs against localized tumor growth and (3) to assess the feasibility of combination therapy, i.e. chemotherapy in combination with ITs. The models to be utilized in this project are a human colon, Ht-29, and ovarian (OVCAR) carcinomas xenografted into nude mice.

RESULTS

1. Animal Models

The initial model developed to explore the feasibility of intracavitary administration of ITs alone or following chemotherapy was a transplantable human colon carcinoma (Ht-29) xenografted intraperitoneally (ip) into athymic mice. Mice injected ip with 3 million ascites cells developed a gradual abdominal distention due to ascites within 18 to 20 days post-tumor cell inoculation. In this xenograft model, untreated mice die in a range of 30 to 40 days. On autopsy, solid tumor as well as large amounts of ascites cells, 50 million, were observed confined to the peritoneal cavity. Furthermore, no evidence of distant metastases was detected by either extensive macroscopic or microscopic analysis of tissue sections. The second animal model under investigation in this project is a human ovarian carcinoma (OVCAR-3) in athymic mice. Following ip injection of 4 million tumor cells, our investigations revealed a localized disease that presented both malignant ascites and solid tumor throughout the peritoneal cavities of mice. Animals die within 50 to 60 days of tumor cell inoculation. Since multiple drug resistance is a problem encountered in the clinical use of chemotherapy, this model is ideal for testing the therapeutic efficacy of intracavitary administration of specific ITs alone or in combination with drug therapy.

2. Drug Therapy

Since the major goal of these studies was to evaluate the efficacy of ITs following cytoreductive therapy, investigations have been underway to identify a clinically active drug that would induce a state of temporary remission (defined as lack of grossly visible ascites in mice before the initiation of IT treatment). The drug initially examined for cytoreductive activity in the Ht-29 model was cyclophosphamide (Cy). Toxicology studies in normal mice revealed that two course of Cy (300 mg/kg) administered ip a week apart resulted in the death of approximately 5% of the mice as well as a 3 to 5 gm body weight loss. WR-2721, a drug reported to prevent bone marrow toxicity induced by Cy, was therefore utilized to determine if this agent would protect mice from the lethal effects of treatment with 300 mg/kg of Cy. Mice were inoculated ip 10 and 17 days post-tumor with 300 mg/kg of Cy. WR-2721, 100 mg/kg, was administered ip one-half hour prior to each Cy treatment. This regimen significantly increased (P=.002) the MST from 35 days in the control groups (range, 28 to 37 days) to 61 days in the drug-treated groups (range, 50 to 63 days). Thus, Cy treatment induced a remission period of approximately 3 weeks. In contrast, the administration of two courses of WR-2721, 100 mg/kg, had no antitumor effect. Finally, co-administration of WR-2721 protected the mice from the lethal effects of Cy alone (300 mg/kg). The drugs of choice utilized against OVCAR-3 were Cy and cis-platinum. Inoculation of $4X10^6$ tumor cells, ip, into mice followed by the ip administration of maximal doses of Cy, 200 mg/kg, and cis-platinum, 5 mg/kg, 21 days post tumor cell inoculation, a time when physical signs of ascites are grossly apparent, resulted in a remission period characterized by a lack of visible ascites. The duration of these remission was approximately five to six weeks with subsequent relapse and death of all treated mice in 90-120 days.

3. In Vivo Antitumor Activity of ITs

Studies were undertaken to evaluate the effects of a mouse anti-human ovarian monoclonal antibody (MoAb) linked to Pseudomonas exotoxin (OVB-3-PE) against an early phase of the colonic disease. Beginning 3 days after Ht-29 injection (3 million cells), mice received either three or six ip injections (0.5 μg) of OVB-3 or OVB-3 (PE). Mice that received three or six treatments of OVB-3 alone had median survival times (MSTs) of 39 and 34 days, respectively, which did not differ significantly from the MST for untreated mice (33 days). In contrast, animals given three or six treatments of OVB-3 (PE) IT experienced a highly significant (P=.002) increase in survival times (MSTs of 62 and 68 days, respectively. Mice treated with an irrelevant IT (anti-Tac PE), .5 μg's, had an MST (31 days) similar to that observed in the control groups. Furthermore, co-injection of unconjugated OVB-3, 20 μg, and OVB-3 (PE), .5 μg, blocked the antitumor of OVB-3 (PE) (MST=38 days). Studies were also undertaken to evaluate another specific MoAb coupled to the same toxin against an early phase of the colonic disease. A mouse anti-human small cell carcinoma MoAb, Nr-Lu-10, was shown to be cross-reactive with Ht-29 via flow cytometry and following linkage to PE was observed in vitro to be highly cytotoxic against Ht-29. Mice that received three or six treatments of Nr-Lu-10 alone exhibited similar MSTs as that seen in the untreated control group. The administration of three or six injections of Nr-Lu-10 (PE), .5 μg, significantly, P=.002, enhanced the survival times of mice (MSTs of 50 and 60 days) respectively, when compared to the untreated control groups (MST=35 days). Finally, co-injection of unconjugated

Nr-Lu-10 (20 μg) and Nr-Lu-10 (PE), .5 μg, blocked the antitumor effect of the specific IT (MST=37 days). Currently, two other ITs, a mouse antihuman colon and transferrin both of which coupled to the A chain of ricin, are under investigation against an early stage of the Ht-29 disease. If either IT demonstrates an antitumor effect against Ht-29, these ITs will be subjected to testing following cytoreductive therapy.

4. Chemo-Immunotoxin Therapy Against Ht-29

Since IT therapy alone increased the life span of mice bearing a minimal tumor burden, investigations were undertaken to explore the therapeutic efficacy of such agents following cytoreductive therapy. After IP administration of 3 million ascites cells on day 0, mice received 300 mg/kg of Cy plus 100 mg/kg of WR-2721 10 and 17 days after tumor cell inoculation. We observed a significant increase in MST (from 37 days to 60 days) in the drug-treated groups compared to the untreated mice (P=0.002). Groups of mice that received two, four or seven treatments of OVB-3 (PE), 0.5 μg, during and after debulking therapy, a time of minimal tumor burden, had further significant increases in MST (P=<0.002) to 81, 87 and 96 days, respectively, compared to the drug treated group (MST=60). Furthermore, a positive trend in significance (P=0.031) was noted with regard to MST and the number of IT treatments administered following cytoreductive therapy. A similar therapeutic response was observed utilizing Nr-Lu-10 (PE). Groups of mice that received two, four or seven treatments of the IT, 0.5 μg, had a significant increase in MSTs (P=<0.002) to 89, 97, and 105 days, respectively, when compared to the drug treated group (MST=59). Finally, the importance of a reduced tumor burden for efficacy of IT treatment was exemplified by the fact that diseased mice that received two or seven treatments of either IT, in the absence of chemotherapy, beginning 14 days post-tumor cell inoculation had MSTs similar to that observed in the untreated control group (MST=36).

PUBLICATIONS

Morgan AC Jr, Pearson JW, Oldham RK. Preclinical evaluation of immunoconjugates. In: Vogel CM, ed. Immunotoxins: Current Methods and Approaches for the Targeting of Toxins and Other Substances. New York: Oxford University Press, 1987;241-258.

Pavanasasivam G, Pearson JW, Bohn WR, Oldham RK, Saydoff J, Morgan AC Jr. Monoclonal antibody-toxin conjugates to a human melanoma associated antigen: Highly potent and selective conjugates of gelonin - Comparison with other A-chain conjugates, Cancer Res 1987;47: 3169-3173.

Morgan AC, Bordonaro J, Pearson JW, Pavanasasivam G. Immunotoxins to a human melanoma-associated antigen: Resistance to pokeweed antiviral protein conjugates J Nat Cancer Inst 1987;78: 1101-1106.

SUMMARY REPORT

LABORATORY OF BIOCHEMICAL PHYSIOLOGY

October 1, 1987 to September 30, 1988

INTRODUCTION

The Laboratory of Biochemical Physiology (LBP) of the BRMP was established in 1986 to conduct research on the genetic and biochemical events related to the development and expression of the malignant phenotype. An important aspect of this mission is to understand the control of cell proliferation/differentiation and to develop strategies for therapeutic intervention in the malignant process based on these findings. We have been applying molecular biology, biochemistry and microinjection techniques to the studies of protein kinase C, phospholipase C, ras proteins and other guanyl nucleotide binding proteins (G proteins) involved in signal transduction pathways, and cytokines/lymphokines.

The human immunodeficiency virus (HIV) has the capability of infecting the immune systems and has been associated with the acquired immunodeficiency syndrome (AIDS). With respect to AIDS-related research, we have been studying the regulation of HIV gene expression and viral latency, and the biosynthetic pathways of HIV envelope proteins. These studies are aimed at the evaluation of possible targets for drug development and immune intervention. The main research activities of the LBP staff during the past fiscal year are summarized below.

STUDIES ON THE BIOLOGICAL FUNCTIONS OF HUMAN RAS PROTEINS

The ras gene family codes for a membrane-associated protein p21 closely related to the G-protein family of the signal transduction pathways. We have studied the role of ras proteins in phosphoinositide turnover and Xenopus oocyte maturation. Our results suggest that normal ras protein and PDGF stimulate PI turnover of NRK cells via different pathways and that oncogenic ras protein is without significant stimulatory effect in this action. These findings are of considerable interest, implying an important regulatory role for ras proteins in cellular signal transduction pathways.

Xenopus oocytes have become a convenient system for biochemical analysis of ras action. Our results indicate that microinjection of oncogenic ras protein induces oocyte maturation and markedly stimulates ribosomal protein S6 phosphorylation in oocytes, whereas normal ras protein is without effect. Furthermore, we have demonstrated that injection of protein kinase C potentiates the induction of S6 phosphorylation and oocyte maturation by insulin or microinjected oncogenic ras protein. Thus, the biochemical pathways of oocyte maturation regulated by insulin receptor, ras proteins, and protein kinase C seem to converge on S6 phosphorylation through intervening steps.

HUMAN GUANYL NUCLEOTIDE BINDING PROTEINS (G PROTEINS) AND CANCER RESEARCH

Go-alpha is a member of the family of G proteins that mediate signal transduction from cell surface receptors to effector enzymes. Different levels of Go-alpha are found in different tissues. Particularly high levels are found in normal brain, other normal and malignant neuronal and neuroendocrine tissues and endocrine cells in the adrenal, pituitary, thyroid and pancreas. Although the function of Go-alpha has not been elucidated, it can interact in vitro with muscarinic receptors, gamma-amino-butyric acid beta receptors, alpha-adrenergic receptors, and rhodopsin. A role in coupling opiate receptors to calcium mobilization has been suggested.

The ADP-ribosylation factor (ARF) is the 21 KDa GTP-binding protein that serves as the cofactor in the cholera toxin catalyzed activation of the regulatory subunit of adenylate cyclase (Gs). ARF is an ubiquitous protein found in every eukaryotic cell examined so far. It is present in high abundance in brain tissue. The physiological role of ARF has not yet been identified.

Human Go-alpha and ARF have been molecularly cloned and characterized in this laboratory. Comparison of the human Go-alpha sequence with bovine and rat Go-alpha shows >90% homology at the nucleotide and deduced amino acid level. There is 100% identity at the amino acid level for the cholera and pertussis toxin-catalyzed ADP ribosylation sites, the putative guanine nucleotide binding, and the GTP hydrolysis sites. Comparison of human ARF sequence with bovine ARF shows >90% homology at the nucleotide level and 100% identity at the deduced amino acid level. The availability of the Go-alpha and ARF cDNA clones will make it possible to pursue structure function studies and the regulation of Go-alpha and ARF expression.

A preliminary report has suggested that Go-alpha is produced in considerable amounts by all types of neuroendocrine tumors but not in noneuroendocrine tumors. In collaboration with Dr. Lee J. Helman, we have examined Go-alpha mRNA expression in various tumor samples. Our results showed that Go-alpha mRNA was expressed in neuroendocrine tumors (e.g. pheochromocytoma, medullary thyroid carcinoma, gastrinoma, glucagonoma, and carcinoid), but not in nonneuroendocrine tumors (e.g. squamous cell cancer of lung, colon cancer and glioblastoma). In contrast, ARF mRNA was expressed ubiquitously in all tumor samples. Thus, the human Go-alpha cDNA clone may provide a useful tool for the diagnosis of neuroendocrine tumors.

EXPRESSION OF CYTOKINES IN HUMAN MELANOMA CELLS

Multiple immunomodulating cytokine activities have been detected in the supernatants of cultured human melanoma cell lines. Although the role of these factors in the biology of melanoma is not yet understood, they could potentially modulate the host immune response to this neoplasm. In collaboration with Drs. Thomas Schwarz and Thomas Luger (University of Vienna), we have examined two human melanoma cell lines, WM-9 and KRFM-2, with Northern blot analysis for the expression of IL-1, GM-CSF, and PDGF,; cytokines which are potent mediators of inflammation. Our results indicated that WM-9 constitutively expressed IL-1-alpha, which was upregulated by PMA, LPS and human rIL-1-alpha. In contrast no IL-1-alpha mRNA was detected in KRFM-2 either constitutively or after PMA, LPS, or IL-1 induction. Neither WM-9 nor KRFM-2 expressed IL-1-beta under the above conditions. We also found that both WM-9 and KRFM-2 constitutively express GM-CSF mRNA which was increased five-fold with PMA induction. Likewise, using the v-sis oncogene probe, homologous to the PDGF beta chain, we found that both WM-9 and KRFM-2 express abundant amounts of PDGF mRNA. Thus, the two human melanoma cell lines, WM-9 and

KRFM-2, demonstrate heterogeneity in IL 1-alpna expression and no IL 1-beta expression, but both expressed GM-CSF and PDGF mRNA. The production and modulation of these melanoma derived factors may be important for host-melanoma immune responses.

INTRACELLULAR ACTIVITIES OF MICROINJECTED CYTOKINES/LYMPHOKINES AND PHOSPHOLIPASE C.

Cytokines and lymphokines bind to plasma membrane receptors on target cells and are internalized by receptor-mediated endocytosis. The biochemical signal generated by this process is transduced into the cell through mechanisms that are not completely understood. Most studies dealing with post-receptor events have focused on second messenger systems such as intracellular calcium, phospholipase C, cAMP dependent protein kinase or C-kinase. We have assessed the direct intracellular role of various cytokines and lymphokines by microinjecting them into the cytoplasm of specific target cells, thus bypassing receptor binding and subsequent internalization. Our results demonstrate that microinjected cytokines such as interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF) are capable of inducing an intracellular response, whereas IL-1 and IL-2 do not have intercellular effects. Murine IFN-gamma treatment of mouse macrophages results in induction of Ia on the cell surface, while human IFN-gamma treatment does not. Species specificity of interferons for cell surface receptors has been well characterized. However, human IFN-gamma, when microinjected into murine macrophage cells, induces the expression of Ia antigen on the cytoplasmic surface of injected and neighboring cells. This suggests that the injected cells responded to injected IFN-gamma by secreting a soluble secondary mediator that subsequently activates 40-50% of the cells in cultures to express Ia. These results demonstrate that the species specificity observed with IFN-gamma is at the cell surface receptor level and that either human or murine IFN-gamma have a functional intracellular effect on Ia gene expression in mouse macrophages.

TNF has pleiotropic activities on various normal and transformed cells, including cytotoxic effects on specific target cells and growth stimulatory effects on other cell types. TNF has no cytotoxic effects on macrophages when treated extracellulary, but when microinjected, the injected cells display signs of toxicity within 10 minutes and die after 3-6 hours. Soluble TNF also kills fibroblasts within 4-8 hours after injection. On the other hand, TNF induces serum-deprived fibroblasts to enter the S phase of the cell cycle before they are killed.

Phosphoinositol-specific phospholipase C (PI-PLC) is an important enzyme in phosphoinositide metabolism leading to the generation of two second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). Similar to oncogenic ras proteins, microinjection of PI-PLC induces serum starved NIH-3T3 cells to enter the S phase of the cell cycle. Bovine brain cDNA encoding PI-PLC has been molecularly cloned. There is a striking sequence similarity between specific regions of PI-PLC cDNA and the non-catalytic domain of the non-receptor tyrosine kinases. The newly characterized crk transforming gene of the avian sarcoma virus CT10 also contains extensive sequence similarities with PI-PLC cDNA. Our microinjection studies clearly demonstrate the growth-promoting activity of PI-PLC.

MOLECULAR CLONING AND SEQUENCE ANALYSIS OF XENOPUS cDNAs ENCODING FOR THE PROTEINS INVOLVED IN SIGNAL TRANSDUCTION

Xenopus oocytes have become an ideal system for the studies of signal transduction. Recently, the cDNAs encoding for protein kinase C, phospholipase C, and various guanyl nucleotide binding proteins have been molecularly cloned from the mammalian species. To identify and characterize these proteins in *Xenopus laevis*, we have isolated cDNA clones encoding Go-alpha, Gi-alpha, Gs-alpha, ADP-ribosylation factor (ARF), *ras*, phospholipase C, and two types of protein kinase C from *Xenopus* oocytes. These cDNA clones have been sequenced. Comparison of the sequences of the coding regions with mammalian cDNAs shows good homology at the nucleotide and deduced amino acid level. The conserved nature of amino acid sequences between *Xenopus* and mammalian species suggests that the proteins involved in signal transduction are important in evolutionary history. We have examined the mRNA expression in *Xenopus* tissues. All of these mRNAs were expressed more abundantly in brain tissue. The expression of these mRNAs at various developmental stages will be determined. The availability of the cDNA clones will make it possible to express these proteins in large quantities using our expression systems, and to pursue structure function studies by site-directed mutagenesis.

MECHANISMS OF ACTION OF THE INTERFERONS

The interferons (IFNs) are a family of proteins with potent antiviral, antiproliferative, and immunomodulatory activities. In order to elucidate the multiple facets in the mechanisms of IFN action, we have been concentrating on the structure and modulation of IFN receptors, studies on signal transduction, and studies of the IFNs as modulators of the expression of specific proteins.

In studies on the structure of the receptors for IFN-alpha, we have developed a ligand blotting technique. This method may prove generally useful for the study of other receptors for which an antibody is not yet available. We have shown that 125I-rIFN-alpha A can bind specifically to two proteins with apparent MW of 105 and 90 KDa. Only the 105 KDa receptor is down-regulated after binding. It is not currently known whether these two IFN binding proteins are distinct polypeptides, or if they are a single type of polypeptide with differing levels of glycosylation.

In studies to elucidate signal transduction mechanisms in the action of IFN-alpha, we have utilized protein kinase inhibitors and have found evidence that a functional protein kinase C is required for induction of 2-5A synthetase mRNA and activity by IFN-alpha. However, phorbol esters, with or without a calcium ionophore, could not mimic the IFN response by induction of 2-5A synthetase activity. These results indicate that a functional protein kinase C is necessary, but that its activation is not sufficient for induction of 2-5A synthetase by IFN-alpha. This suggests that multiple signal transduction pathways may be required in the action of IFN.

In studies of the IFNs as modulators of the expression of cellular proteins, we have found that rIFN-gamma treatment of myelomonocytic cell lines and peripheral blood monocytes, but not T lymphocytes, decreases expression of CD4 which is the receptor for HIV. Since monocytes/macrophages are considered to be an important reservoir for HIV in vivo, the effect of IFN-gamma to decrease CD4 on these cells may have clinical relevance for the treatment of AIDS.

BIOCHEMICAL AND MOLECULAR STUDIES OF HUMAN IMMUNODEFIENCY VIRUS (HIV)

Recently, the laboratory has initiated studies on the elucidation of HIV *env* protein glycosylation and proteolytic processing pathways. The aim of this project is to develop strategies for the therapeutic intervention of AIDS. This work will be performed by the newly recruited scientist, Dr. Wen-po Tsai, who had extensive experience in retrovirology. We are now in the process of obtaining HIV virus, human cell lines, and specific antibodies against HIV *env* proteins. With the help of Dr. Peter Nara, we have set up an HIV infectivity assay. The assay will be very useful for screening potential therapeutic drugs.

In molecular studies of HIV, we have been investigating the role of trans-activator (tat-III) on HIV gene expression and viral latency. The HIV retrovirus encodes a trans-activator (tat-III) of 14 KDa that markedly increases the expression of HIV genes. There was no direct evidence to indicate transcriptional activation of HIV by tat-III. Using a modified in vitro transcription system and a gel retardation assay, we have obtained direct evidence that tat-III stimulates transcription specifically from the HIV-LTR. This activation is primarily involved in the formation of the transcription preinitiation complex. The in vitro studies will provide us with a better understanding of the mechanism of regulation of HIV gene expression and viral latency, and thus can provide a strategy for the control of the virus infection and its propagation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09299-01 LBP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Glycosylation and Proteolytic Processing of Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* (Name, title, laboratory, and institute affiliation)

PI:	H. Kung	Chief	LBP, NCI
Others:	W. Tsai	Microbiologist	LBP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Physiology

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
0.5	0.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have initiated the following studies aimed at the evaluation of possible targets for drug development and immune intervention:

1. Elucidation of HIV env protein glycosylation pathways and evaluation of agents that may affect directly or indirectly the glycosylation and consequently the infectivity of HIVs.

2. Characterization of the proteolytic cleavage events of the HIV env gene encoded precursor polyproteins, identification of the proteolytic enzyme(s), and evaluation potential enzyme inhibitors.

PROJECT DESCRIPTION

PERSONNEL

Hsiang-fu Kung	Chief	LBP, NCI
Wen-po Tsai	Microbiologist	LBP, NCI

INTRODUCTION

Retroviruses contain two principle envelope proteins, the surface glycoprotein and the transmembrane protein. These two mature proteins are derived from a single large precursor polyprotein encoded by the env gene. The precursor polyproteins are initially cotranslationally glycosylated in the rough endoplasmic reticulum. They are further processed by additional glycosylation and proteolysis and transported to the plasma membrane where the cleaved products are incorporated into budding viruses. Inhibition of glycosylation and proteolytic processing of the env gene encoded precursor polyprotein may stop the full expression of viral infectivity. We will perform biochemical and immunological studies of HIV env proteins in order to obtain a better understanding of HIV glycosylation pathways and their relation to the viral infectivity.

MAJOR FINDINGS

Novel glycosylation pathways of retroviral proteins have been identified with avian reticuloendotheliosis virus (see publications). The findings will be useful for the biochemical and immunological studies of HIV.

OBJECTIVES AND FUTURE PLANS

The intracellular glycosylation and proteolytic cleavage of human immunodeficiency virus appear to be potential target events for therapy. We propose the following studies:

1. elucidation of HIV env protein glycosylation pathways and evaluation of agents that may affect directly or indirectly the glycosylation and consequently the infectivity of HIVs.

2. characterization of the proteolytic cleavage events of the HIV env gene encoded precursor polyproteins, identification of the proteolytic enzyme(s), and evaluation potential enzyme inhibitors.

PUBLICATIONS

Tsai WP, Copeland TD, Oroszlan S. Biosynthesis and chemical and immunological characterization of avian reticuloendotheliosis virus env gene-encoded proteins. Virology 1986;155:567-83.

Tsai WP, Oroszlan, S. Novel glycosylation pathways of retroviral envelope proteins identified with avian reticuloendotheliosis virus. J Virol, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09300-02 LB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Microinjection of Cytokines, Lymphokines, Phospholipase C, Mutant Ras Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Kung	Chief	LBP, NCI
Others:	S. Durum	Senior Staff Fellow	LMI, NCI
	H. Young	Expert	LMI, NCI

COOPERATING UNITS *(if any)*

BCDP, PRI, NCI-FCRF (M. Smith, J. Keller)

LAB/BRANCH
Laboratory of Biochemical Physiology

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Specific target cells have been microinjected with cytokines and lymphokines in order to study differences between receptor-mediated signal transduction and direct intracellular introduction of these immunoregulators. Species specificity of interferons for cell surface receptors has been well characterized. However, injected human IFN-gamma was shown to have an intracellular function in mouse macrophages. Microinjection of IFN-gamma induced the expression of Ia antigen on the surface of macrophage cells. The IFN-gamma induced expression of Ia was shown to be mediated by a second messenger, GM-CSF. Microinjection of TNF-alpha has been shown to induce killing of macrophages and fibroblast cells on a dose and time dependent fashion.

Certain mutant ras proteins that do not bind GTP were shown to induce DNA synthesis in serum deprived NIH 3T3 cells, suggesting that GTP binding does not necessarily correlate to oncognisity of certain mutant ras proteins.

Phospholipase C (PLC), a central enzyme involved in phospholipid metabolism, has been shown to induce DNA synthesis in serum deprived NIH 3T3 cells at catalytic concentrations of protein. This result directly supports the theory that PLC is an important regulator of signal transduction.

PROJECT DESCRIPTION

PERSONNEL

Hsiang-fu Kung	Chief	LBP, NCI
Scott Durum	Senior Staff Fellow	LMI, NCI
Howard Young	Expert	LMI, NCI

INTRODUCTION

Microinjection techniques have been used for the studies of the biological activities of physiological important molecules such as cytokines lymphokines and enzymes. Direct injection, via glass capillary tube, of purified molecules into living cells is a reasonable and effective way to study the biological activities of purified DNA and proteins on appropriate target cells. The research presented in this report uses microinjection as a tool to study the biological mechanisms of important biological response modifiers such as interferon and tumor necrosis factor. We have established an assay using macrophage cells. This assay has demonstrated that IFN-gamma can elicit some of its biological activities via the second messenger, GM-CSF. Macrophage cells, which synthesis TNF-alpha and are insensitive to extracellular treatment of this cytokine, are killed by microinjecting 100 molecules of TNF intracellularly.

OBJECTIVES

To study the biological activities of cytokines and lymphokines after microinjection into appropriate target cells and to compare the intracellular biological response to the normal biological response observed when ligand binds to its cytoplasmic surface receptor. These studies directly examine the effects on target cells if the normal receptor binding and subsequent membrane signal transduction pathways are bypassed by microinjection. Injection allows for the assay of intracellular activities of cytokines and lymphokines.

To study the biological ramifications of microinjection of phospholipase C into resting fibroblast cells. Phospholipase C, an important enzyme of phosphoinositide metabolism, cleaves phosphatidylionositol 4,5-bisphosphate to generate two growth promoting second messengers, inositol triphosphate and diacylglycerol.

To determine if bacterially expressed mutant ras proteins, which do not bind GTP, are biologically active and able to induce transformation of serum starved fibroblast cells.

METHODS

Microinjection, cell biology and tissue culture, protein purification, immunological staining, and in situ hybridization.

MAJOR FINDINGS

Human interferon-gamma (IFN-gamma), when microinjected into murine macrophage cells, induces the expression of Ia antigen on the cytoplasmic surface of injected and neighboring cells. This result demonstrates that the species specificity observed with IFN-gamma is a result of binding of this ligand to its cytoplasmic surface receptor, and that internalized IFN-gamma has a biological role within the cell. When 200 macrophage cells are injected with 300-600 molecules of IFN-gamma, 250,000 macrophage cells in that culture responded by expressing Ia. This observation suggests that the IFN-gamma injected cells are induced to synthesis and secrete a secondary mediator of the IFN-gamma signal and this mediator in turn activates 40-50% of the macrophage population to express Ia. Neutralizing monoclonal antibody against murine GM-CSF when added to the culture media of IFN-gamma injected cells blocks Ia antigen expression. In addition, this antibody will also partially block Ia expression in macrophage cells treated with soluble murine IFN-gamma. In our microinjection assay GM-CSF is the secondary mediator of the IFN-gamma signal.

GM-CSF was shown by injection assay and by FACS analysis to induce Ia expression on murine macrophage cells. GM-CSF mRNA was shown by in situ hybridization and Northern blot to be induced in macrophage cells by IFN-gamma treatment. GM-CSF was also shown by bioassay to be present in conditional media from IFN-gamma treated and microinjected macrophage cells.

Microinjection of TNF-alpha (100 u/ml) resulted in the killing of target cells within four hours. TNF like molecules are known to be involved in the cell mediated cytoloysis of NK, CTL, and LGL killing of certain tumor cells. Recombinant TNF injected into stationary phase NIH 3T3 cells or macrophage cells induces a rapid cytolytic effect.

Serum depleted NIH 3T3 cells are induced to enter the DNA synthesis phase of the cell cycle by microinjection of phospholipase C I and II. This protein is responsible for the enzymatic hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol-triphosphate and diacylglycerol, which act as second messengers for the growth promoting signal transduction of several polypeptide hormones. PLC at 1 mg/ml is cytotoxic and dissolves the cell membrane, while at 100 ug/ml a 25-fold enhancement of ^{3}H-thymidine incorporation was observed in injected cells compared to noninjected cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Cytokines and lymphokines, as immunomodulators, play a central role in the immune response that protects animals from diseases of all types. Research evidence suggests that many of these immunoregulators may be involved in natural tumor absorption or killing processes. Recombinant cytokines and lymphokines are currently being used in clinical trials with human cancer patients; IFN-alpha has been shown to be active against hairy cell leukemia. The determination of the mechanisms by which cytokines and lymphokines exert their immunomodulatory effects is central to an understanding of their anti-tumor potential. Microinjection has demonstrated that IFN-gamma, in addition to activating cell surface receptors, has an intracellular function. Recent evidence suggests that IFN-gamma itself may be inducing expression of genes within the nucleus of the cell. In order to better understand the role played by cytokines and lymphokines in cell-mediated killing processes, it is necessary to evaluate the biochemical mechanisms activated by these immunoregulators.

GOALS

To continue microinjecting cytokines and lymphokines into appropriate target cells in order to study the differences between extracellular and intracellular delivery of these factors. To use the macrophage assay to study specific events of membrane signal transduction. (In preliminary studies microinjected PLC has been shown to induce Ia expression similar to injected IFN-gamma.)

To complete injection experiments with TNF and study the killing parameters of TNF, lympotoxin, and granules from NK cells to further evaluate the role of these toxins and evaluate cofactor requirements involved in cell mediated cytolysis.

PUBLICATIONS

Willumsen BM, Kung HF, Johnsen M, Lowy DR. A mutation analysis of ras function. Viral Carcinogenesis 1987;24:385-400.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09301-02 LBP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Studies on the Biological Function of Human Ras Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Kung	Chief	LBP, NCI

COOPERATING UNITS *(if any)*

BCDP, PRI, NCI-FCRF (T. Kamata, S. Kelley, M. West)

LAB/BRANCH

Laboratory of Biochemical Physiology

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	0.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oncogenic mutant ras proteins have less GTPase activity and are more potent in inducing cellular DNA synthesis as compared to their normal counterpart. By analogy with G-proteins, ras proteins have been postulated to be involved in the control of growth factor receptor-mediated signal transduction, although the biochemical pathway influenced by ras proteins has not been fully understood. Using *Xenopus* oocytes as a model system, we investigated the possible involvement of ras proteins in the pathway leading to phosphorylation of the ribosomal S6 protein, which is related to the initiation of protein synthesis.

Our results indicate that (1) microinjection of oncogenic T24 ras protein markedly stimulates S6 phosphorylation on serine residues in oocytes whereas normal ras protein is without effect, and (2) injection of anti-ras monoclonal antibody Y13-259 inhibits insulin-induced S6 phosphorylation suggesting that ras proteins mediate insulin-induced S6 phosphorylation. In another set of experiments, we have demonstrated that injection of protein kinase C promotes the induction of S6 phosphorylation and oocyte maturation by insulin or oncogenic ras protein.

These findings are extremely interesting, implying that biochemical pathways regulated by an insulin receptor, ras, and protein kinase C seem to converge on S6 phosphorylation.

PROJECT DESCRIPTION

PERSONNEL

Hsaing-fu Kung	Chief	LBP, NCI

OBJECTIVES

The objectives of this project are to study the biological functions of human normal or oncogenic ras protein in receptor-mediated signal transduction.

MAJOR FINDINGS

It has been suggested that ras proteins play a role in membrane signal transduction by growth factors. However, the exact nature of biochemical pathway regulated by ras proteins remains an enigma. Therefore, we decided to study a ras-controlled biochemical pathway using Xenopus oocytes as a model system.

1. We first investigated the involvement of ras proteins in the pathway leading to phosphorylation of ribosomal S6 protein. It is believed that S6 phosphorylation plays a role in the initiation of protein synthesis and is an early event preceding oocyte maturation. Our results show that microinjection of oncogenic T24 H-ras protein markedly enhances S6 phosphorylation on serine residues in oocytes, whereas normal ras protein is less potent in this activity. This suggests that oncogenic ras protein constitutively activates the phosphorylation of S6 protein.

2. Since it is known that the induction of oocyte maturation by insulin is blocked by anti-ras antibody (Y13-259), we examined the effect of the antibody on S6 phosphorylation in insulin-treated oocytes. We have found that injection of the anti-ras antibody inhibited insulin-induced S6 phosphorylation.

3. Recent studies from other laboratories show that the level of diacylglycerol, an endogenous activator of protein kinase C, is increased following the introduction of the oncogenic ras protein into Xenopus oocytes. To test whether protein kinase C affects a ras protein-regulated pathway, oocytes were injected with protein kinase C either alone or in combination with oncogenic T24 ras protein. Our data indicate that the induction of oocyte maturation and S6 phosphorylation by the ras protein was promoted by co-injection of protein kinase C. Similarly, insulin-induced S6 phosphorylation and oocyte maturation were promoted by injection of protein kinase C. Together, these results suggest that protein kinase C is involved in the insulin and/or ras protein-mediated pathway.

FUTURE PLANS

Since ras proteins bind GTP/GDP and lack phosphotransferase activity, it is possible that a serine kinase or a serine phosphatase activity is regulated by ras guanine nucleotide exchange activity directly or through a cellular factor(s). In our future studies, we will purify the factor(s) involved in S6 phosphorylation and characterize its biochemical and biological properties.

PUBLICATIONS

Kamata T, Kung HF. Effects of ras proteins and PDGF on phosphoinositide turnover in NRK cells. Proc Natl Acad Sci USA, in press.

Brandt-Rauf P, Pincus M, Carty R, Lubowsky J, Avitable M, Kung HF, Maizel J. Conformational effects of amino acid substitutions at position 10, 12 and 13 in the p21 protein. J Prot Chem, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09302-02 LBP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

G-Proteins and Signal Transduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Lavu	Senior Staff Fellow	LBP, NCI
Others:	H. Kung	Chief	LBP, NCI
	J. Clark	Senior Staff Fellow	BTB, NCI
	J. Moss	Deputy Chief	LCM, NCI
	M. Vaughan	Chief	LCM, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Physiology

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Guanyl nucleotide binding proteins (G-proteins) are critical components of several regulatory pathways in animal cells, specifically involved in signal transduction. Recent evidence suggests the involvement of G-proteins in the regulation of various BRM's and alterations in their function may contribute to the pathogenesis of disease. Of these G-proteins, Go is present in large quantities in critical organs like brain and heart. Its function is not known. Hence, these studies are aimed at understanding the role of this protein in various signal transduction pathways and its role in various malignancies associated with brain. A bovine retinal cDNA clone, encoding the complete alpha subunit of Go, was isolated and sequenced, which encodes for 354 a.a.'s polypeptide (Mr 39,900). Comparison of this G-protein with other G-proteins have revealed good homology at important critical sites like GTP binding, GTP hydrolysis, and at sites ADP ribosylated by cholera toxin and pertussis toxins. Using the cDNA clone as a probe, genomic clones coding for Go-alpha were obtained and characterized. Eight clones encompassing 50Kb, coding for the 5' part and middle part of the coding region were characterized, and the first 5 exons were sequenced. Using the 3' probe, we have obtained two more clones, and we are currently analyzing them. Also, using the bovine Go-alpha cDNA clone as a probe, we have obtained cDNA clones from a normal human brain library. The cDNA clones are sequenced and found to have strong homology to the bovine cDNA clone. As the 5' part of the clone is missing, we obtained genomic clones of the corresponding region and characterized the first two exons corresponding to the missing part.

PROJECT DESCRIPTION

PERSONNEL

Sukadev Lavu	Senior Staff Fellow	LBP, NCI
Jeff Clark	Senior Staff Fellow	CRB, NCI
Joel Moss	Deputy Chief	LCM, NCI
Martha Vaughan	Chief	LCM, NCI
Hsiang-fu Kung	Chief	LBP, NCI

INTRODUCTION

Guanyl nucleotide binding proteins (G-protein) are critical components of several regulatory pathways in animal cells, specifically involved in signal transduction. Recent evidence suggests that G-proteins may play a role in mediating the signal transduction of various BRM's, and that any alteration in their function may contribute to the pathogenesis of disease. (Evans et al., 1987, Science 325,166) (Growler et al., 1987, Nature 327,229) The product of the ras oncogene is also a G-protein. Mutations of this gene appear to be important in oncogenesis, since they are found at increased frequency in a variety of human malignancies. G-proteins are a multi-gene family with alpha, beta and gamma subunits. Beta and gamma subunits of G-proteins appear to be similar but alpha subunits are unique to each G-protein.

OBJECTIVES

Go is present in large quantities in critical organs, especially vertebrate brain and heart. Its function, at this time, is not known. Hence, these studies are aimed at understanding the role of this protein in various signal transduction pathways and its possible role in various neuronal malignancies.

METHODOLOGY

1. DNA extraction and restriction enzyme analysis.
2. Southern blotting, Northern blotting and hybridization.
3. Making genomic libraries, cDNA libraries, and screening for recombinant clones.
4. Detailed characterization of clones.
5. Nucleotide sequence analysis.

MAJOR FINDINGS

A bovine retinal cDNA clone encoding the complete alpha subunit of Go was isolated and sequenced, and found to encode for 354 a.a.'s polypeptide (Mr 39,900). Comparison of this G-protein with other G-proteins has revealed close homology at important functional sites (GTP binding, GTP hydrolysis, and at sites ADP ribosylated by cholera toxin and pertussis toxins). In order to obtain large quantities of Go-alpha for structure function studies, we are in the process of expressing this gene in bacteria and mammalian cells.

Using the cDNA clone as probe, genomic clones coding for Go-alpha were obtained and characterized. Eight clones encompassing 50Kb, coding for the 5' part and the middle part of the coding region were characterized, and the first 5 exons were sequenced. Using a 3' specific probe, two more clones were obtained, and the detailed characterization of these clones are underway.

Also, using the bovine Go-alpha cDNA clone as a probe, we have obtained cDNA clones from a human brain, Alzheimer's tissue, and Neuroblastoma. The cDNA clones from the normal human library are sequenced and found to have a polypeptide of 305 amino acids, but it lacks the 5' region of 49 amino acids. Presently, we are screening the library again with a 5' specific probe to obtain the full length clone. As the screening was futile, we screened the human genomic library constructed in Lamda EMBL-3 with 5' probe and obtained clones with the first two exons and characterized the entire coding region to human Go-alpha gene. This gene shows approximately 98% identity with bovine and rat Go-alpha genes at the amino acid level and approximately 94% homology at the nucleotide level. Preliminary characterization of the cDNA clones from the Alzheimer's tissue and from the Neuroblastoma library revealed identical nucleotide sequence at the 5' part of the normal human Go-alpha gene. Detailed characterization of these cDNA clones is underway.

FUTURE PLANS

Studies are planned to express these cDNA clones in mammalian cells and in bacteria for the structure function studies of Go-alpha. Site-directed mutagenesis will be employed for these studies.

The genomic clone will be used in studying the regulatory elements of the gene and to look at potential alternative splicing events which have been reported for human Gs-alpha clones.

PUBLICATIONS

Schultz AM, Tsai SC, Kung HF, Oroszlan S, Moss J, Vaughan M. Alkali-stable linkage of myristic acid in Go-alpha. Biochem Biophy Res Comm 1987;146:1234-239.

Lavu S, Clark J, Swarup R, Matsushima K, Paturu K, Moss J, Kung, HF. Molecular cloning and DNA sequence analysis of the human guanine nucleotide-binding protein Go-alpha. Biochem Biophy Res Comm 1988;150:811-15.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09312-02 LBP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Action of the Interferons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Kung	Chief	LBP, NCI
Others:	G. Princler	Chemist	LBP, NCI
	M. Schwabe	Visiting Fellow	LBP, NCI
	L. Varesio	Visiting Scientist	LMI, NCI

COOPERATING UNITS *(if any)*

BCDP, PRI, NCI-FCRF (C. Faltynek); BCDP, PRI, NCI-FCRF (D. Radzioch)

LAB/BRANCH
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2.0	0.0	2.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In studies of the structure of the receptors for interferon-alpha (IFN-alpha) we have utilized covalent cross-linking methods and a ligand blotting technique, which we recently developed. The ligand blot is analogous to a Western blot, but it utilizes a radiolabeled ligand instead of an antibody. This method may prove generally useful for the study of other receptors for which an antibody is not yet available. Using these techniques, we have found that 125I-rIFN-alpha A can bind specifically to two proteins with the apparent MW of 105 and 90 KDa. Studies using intact cells have demonstrated preferential down regulation of the 105 KDa receptor. Covalent cross-linking studies have also revealed the existence of a higher MW complex of approximately 240 KDa, which may represent the specific association of the IFN-binding protein with an intracellular protein. These studies reveal a greater complexity to the IFN-alpha receptors than was previously recognized. The relationship between these IFN-receptor complexes and responses to IFN are being examined.

In studies to elucidate signal transduction mechanisms in the action of IFN-alpha, we have utilized protein kinase inhibitors. We have found evidence that a functional protein kinase C is required for induction of 2-5A synthetase mRNA and activity by IFN-alpha. However, protein kinase C activation alone is not sufficient for induction of 2-5A synthetase, suggesting that multiple signal transduction pathways may be required in the action of IFN.

In studies of the IFNs as modulators of the expression of cellular proteins, we have found that rIFN-gamma treatment of myelomonocytic cell lines and peripheral blood monocytes, but not T lymphocytes, decreases expression of CD4, the receptor for HIV. Since monocytes/macrophages are considered to be an important reservoir for HIV in vivo, the effect of IFN-gamma in decreasing CD4 on these cells may have clinical relevance for the treatment of AIDS.

PROJECT DESCRIPTION

PERSONNEL

Hsiang-fu Kung	Chief	LBP, NCI
Gerald Princler	Chemist	LBP, NCI
Michael Schwabe	Visiting Fellow	LBP, NCI
Luigi Varesio	Visiting Scientist	LMI, NCI

OBJECTIVES

The interferons (IFNs) are a family of proteins with potent antiviral, antiproliferative, and immunomodulatory activities. Considerable knowledge has been gained in recent years concerning the biochemical mechanisms in the action of the IFNs. Nevertheless, there are many aspects that are still poorly understood. The objectives of this project are to further elucidate the multiple facets in the mechanisms of IFN action. Current work emphasizes studies on the structure and modulation of IFN receptors, studies on signal transduction, and studies of the IFNs as modulators of the expression of specific proteins.

MAJOR FINDINGS

Studies by several investigators using radiolabeled IFNs have established that all the IFN-alpha and IFN-beta subspecies can compete for binding to a common cell surface receptor, whereas IFN-gamma binds to a separate receptor. However, it is not known whether greater complexity exists for the IFN receptors. We have been addressing the questions of whether or not there are multiple types of receptors for IFN-alpha, if the receptors on various cell types show structural differences that may account for differing sensitivities to IFN-alpha, and if there is any evidence for multiple subunits of the IFN-alpha receptor or specific association of the IFN receptor with other cellular proteins. To address these questions we have used two types of techniques. In the first technique, 125I-labeled IFNs are allowed to bind to cell surface receptors on intact cells. The IFN is then covalently linked to its receptor using a bifunctional crosslinking agent, followed by cell lysis, SDS-PAGE, and autoradiography. The second technique utilizes a ligand blot method, analogous to a Western blot, that we have developed to study IFN receptors. In this method, either cell extracts or purified plasma membranes are subjected to SDS-PAGE, followed by blotting onto nitrocellulose and probing with 125I-labeled IFN. This latter method is novel in the field of polypeptide hormone receptors and provides a mechanism to directly study receptors for which no antibody is yet available. The method may prove to be generally applicable to the study of other polypeptide hormone receptors.

Using these techniques, we have found that rIFN-alpha A can bind to two proteins with apparent MW of 105 and 90 KDa on Daudi cells. The binding to both proteins is specific, since unlabeled rIFN-alpha A and beta, but not rIFN-gamma, prevents the binding of 125I-IFN-alpha A. Studies using intact cells have demonstrated preferential down regulation by rIFN-alpha A of the 105 KDa receptor. It is not currently known whether these two IFN binding proteins are distinct polypeptides or are a single type of polypeptide with differing levels of glycosylation. Covalent cross-linking studies also revealed the existence of a higher MW complex of approximately 240 KDa. This complex was only observed when membrane permeable but not membrane impermeable cross-linking agents were utilized. This suggests that the high MW complex may represent the specific association of the IFN-binding protein with an intracellular protein.

Currently, the signal transduction mechanisms in the action of the IFNs are poorly understood. Yap and coworkers have reported (Science 234:355,1986) that treatment of the Daudi B lymphoblastoid cell line with IFN-alpha or -beta results in a rapid, transient increase in the level of diacylglycerol, a potent activator of protein kinase C. To assess the role for protein kinase C in the IFN response, we have examined the effects of protein kinase inhibitors upon the induction of 2-5A synthetase by rIFN-alpha A in Daudi cells. At 20 uM, the protein kinase inhibitor H7, which is a potent inhibitor of both protein kinase C and cyclic nucleotide dependent protein kinases, almost completely inhibits induction of both 2-5A synthetase mRNA and activity. In contrast, at 20 uM the protein kinase inhibitor HA1004, which is a potent inhibitor of the cyclic nucleotide dependent protein kinases, but is a weak inhibitor of protein kinase C, has no effect on induction of 2-5A synthetase mRNA or activity. These results suggest that functional protein kinase C, but not cyclic nucleotide dependent protein kinases, is required for the action of IFN-alpha in Daudi cells. Inhibition of 2-5A synthetase induction by H7 was not due to inhibition of the binding or internalization of rIFN-alpha A. At the concentrations of H7 used in the above experiments ($\leq$20 uM), the inhibition of 2-5A synthetase induction could not be attributed to generalized inhibition of macromolecular synthesis. Experiments in which cells were pretreated with rIFN-alpha A, followed by removal of the IFN and subsequent incubation with or without H7, suggested a requirement for protein kinase C in the action of IFN not only at early time points to induce the 2-5A synthetase mRNA, but also later to express the 2-5A synthetase activity. However, phorbol esters, with or without a calcium ionophore, could not mimic the IFN response by induction of 2-5A synthetase activity. These results indicate that a functional protein kinase C is necessary, but that its activation is not sufficient for induction of 2-5A synthetase by IFN-alpha. Moreover, these results suggest that multiple signal transduction pathways may be operative subsequent to the IFN-receptor interaction.

There is considerable evidence that the CD4 cell surface glycoprotein is the receptor for the AIDS virus HIV-1 on both T lymphocytes and monocytes. Because the IFNs are potent modulators of the expression of several cellular proteins, we have examined whether or not the IFNs modulate the expression of the HIV-1 receptor on T lymphocytes and monocytes as part of their antiviral mechanism. We have found that rIFN-gamma decreases by 50% the expression of CD4 on the promonocytic cell line U937. When freshly isolated peripheral blood monocytes were incubated in vitro for 48 hours, CD4 levels increased; treatment with rIFN-gamma at concentrations as low as 2 U/ml prevented this increase. Moreover, treatment with rIFN-gamma reduced the percentage of CD4 positive peripheral blood monocytes 2-5-fold. The effect of IFN-gamma to decrease CD4 expression appeared to be specific to cells of the myelomonocytic lineage, since treatment with rIFN-gamma had no effect on CD4 expression on peripheral blood T lymphocytes or T cell lines. Because monocytes/macrophages are considered to be a major reservoir for HIV-1 in vivo, the effect of IFN-gamma to decrease CD4 expression on monocytes may have clinical relevance to the treatment of AIDS.

PUBLICATIONS

Faltynek CR, Baglioni C. Interferon induction of the antiviral state: early events. Mechanisms of interferon actions. Pfeffer, LM (Ed) CRC Press, Boca Raton, Florida, 1987;99.

Faltynek CR. Opposite effects of recombinant interferon alpha A and deoxycoformycin on adenosine deaminase activity in the Daudi B lymphoblastoid cell line. Blood 1988;71:59-64.

Elliott KRF, Princler GL, Urba WJ, Faltynek CR. Synergistic antiproliferative effects of glucocorticoids and interferon-alpha on some lymphoid cell lines. J Cell Physiol 1988;134:85-92.

Faltynek CR, Princler GL, Ruscetti FW, Birchenall-Sparks M. Lectins modulate the internalization of recombinant interferon-alpha A and the induction of 2'5'-oligo(A) synthetase. J Biol Chem 1988;263:7112-117.

Faltynek CR, Oppenheim JJ. Interferons in host defense. J Natl Cancer Inst 1988;80:151-153.

Honda S, Asano T, Kajio T, Nakagawa S, Ikeyama S, Ichimori Y, Sugino H, Nara K, Kakinuma A, Kung H. Differential purification by immunoaffinity chromatography of two carboxy-terminal portion-deleted derivatives of recombinant human interferon-gamma from E. coli. Jnl Interferon Res 1987;7:145-54.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09315-01 LBP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of HIV Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Kung	Chief	LBP, NCI
Others:	F. Ruscetti	Microbiologist	LMI, NCI

COOPERATING UNITS *(if any)*
PRI, NCI-FCRF (Raziuddin)

LAB/BRANCH
Laboratory of Biochemical Physiology

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	0.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gene expression of human immunodeficiency virus (HIV) is enhanced by a trans-activator, the tat-III protein. There is no direct evidence to indicate transcriptional activation of HIV expression by tat-III. Sensitive in vitro transcription and DNA-binding gel retardation assays have been devised to study the molecular mechanism of HIV gene regulation in vitro. Using the in vitro transcription system, we have demonstrated that the extracts obtained from cells, which produce tat-III, selectively stimulate steady state levels of the transcript from the HIV long terminal repeat (LTR) promoter. The DNA-binding gel retardation assays further demonstrate a stable activated preinitiation complex, formed either by direct binding of tat-III to DNA elements of the HIV-LTR or indirectly, by some other cellular transcription factor(s) mediated and/or induced by tat-III. This data implicates transcription as the site of tat-III action in trans-activation phenomenon.

These studies have been further extended to include investigations of the mechanisms of HIV latency in monocytes. Since the monocytes are in the latent (no trans-activation) stage, it is rather difficult to observe expression of the gene at the transcriptional level. Therefore, the latent cells were superinfected by CMV virus or treated with phorbol ester (PMA) to overcome the latency. In vitro transcription, nuclear run-off assays, and Northern analyses of these latently and productively infected monocytes are in progress.

PROJECT DESCRIPTION

PERSONNEL

Hsiang-fu Kung	Chief	LBP, NCI
Frank W. Ruscetti	Microbiologist	LMI, NCI

INTRODUCTION

The human immunodeficiency virus (HIV) is the causative agent of AIDS. The infectious viruses show a remarkably varied repertoire of mechanisms for altering or dominating gene expression in host cells. HIV shows a limited cell range and an internally regulated complex pattern of latency and activation, which usually begins with a prolonged latent period without any virus induced cytopathic effect. Latency can be overcome, however, by various antigenic activators with a resulting burst of virus replication. At present, the mechanism of release from latency is not clear, although the HIV trans-activating gene product tat-III is thought to be directly involved. As in HIV infected cells, tat-III markedly increases HIV gene expression directed by the long terminal repeat (LTR) sequences of the virus.

There is considerable evidence that monocytes and macrophages play a major role in the propagation and pathogenesis of HIV infection. The major cell types infected with HIV in the brain are monocytes and macrophages, leading to the neuropsychiatric abnormalities. The HIV infection which starts with a prolonged latency can be overcome by certain biological as well as chemical inducing agents in vitro.

OBJECTIVES

1. To understand the molecular mechanism and the precise role and action of the trans-activator (tat-III) in HIV gene expression at the transcriptional level.

2. To investigate the mechanism of HIV latency in monocytes which may lead to the development of a sensitive bioassay to quantitate the HIV gene expression in latent as well as restricted infection of the virus.

METHODS

1. In vitro transcription assay:

In order to study the mode of action of tat-III, we have pursued the more direct approach of exploiting a mouse cell line. This line exclusively produces tat-III (in the absence of any other HIV proteins) for use in a modified in vitro transcription system prepared from Hela cells.

Template-DNA from the recombinant plasmid pL3CAT, (Science, 234, 988, 1986), which contains the LTR promoter enhancer as well as downstream TAR sequences, were gel purified from different restriction sites. Hela nuclear extracts were prepared according to the standard method (Cell 36, 357, 1984), whereas the whole cell extracts from control mouse (-tat-III) cell line BXL40 and mouse tat-III producing cell line B2MX3 (Science 234, 988, 1986) were prepared by another published method (Methods Enzymol. 101, 568, 1983).

2. DNA-binding gel retardation assays:

To determine whether transcriptional activation/stimulation phenomena is a direct consequence of the tat-III action, DNA-binding gel retardation assays were performed. The assays used the tat-III producing and (-tat-III) control cell extracts and a DNA probe from the entire LTR 5'- to the transcription start site. The gel-retardation assay was a modification of a previously described method (Cell, 48, 79, 1987).

3. Nuclear run-off assays:

Transcriptional analyses of latently infected monocytes were performed by nuclear run-off assays. THP1, a mature monocyte leukemic cell line, was infected with HIV-1 from an ongoing T-cell isolate. After initial low level productive infection (approximately 10 days post infection period), the cells became latent. These latently infected cells become productive by PMA treatment, as well as by CMV coinfection (Dr. Frank Ruscetti, NCI-BRMP).

Nuclei were isolated from the controls, latent, and productive monocytes. Nuclear run-off assays were performed and hybridized to HIV-LTR as described (Nature 311, 433, 1984).

MAJOR FINDINGS

Gene activation generally requires protein interaction with cis-acting specific regulatory DNA sequences. Consistent with this model for tat-III, using a modified in vitro transcription system prepared from Hela cells, we have demonstrated that an extract obtained from the mouse cells that specifically produce the tat-III protein that selectively stimulates transcription. This activation is primarily involved in the initiation of transcription from the HIV-LTR promoter sequences.

To observe whether or not this activation/stimulation phenomena is a direct consequence of the tat-III action, DNA-binding gel retardation assays were performed with the mouse (-tat III) control and mouse tat-III producing extracts. This process employed the use of a DNA probe from the entire LTR 5' to the transcription start site. A detectable complex was formed with the tat-III producing extract which, in the presence of a Hela cell nuclear extract, showed much greater stimulation and specificity. This indicated that the mouse extracts were limiting in protein factor(s) involved in the complex formation.

The present studies demonstrate, for the first time, a stimulated and active DNA-protein complex in the form of a stable preinitiation complex. There appeared to be a possible tat-III involvement in this process as well. A simple interpretation of these results is that, possibly in combination with a cellular factor, viral tat-III protein acts to enhance transcriptional initiation. It remains unclear, however, whether tat-III involvement is direct or if some cellular transcriptional factor(s) activity is being induced in its presence, as has been reported for adenovirus trans-acting transcriptional activator E1A. The strong basic nature (arg/lysine contents) in addition to a cluster of cysteine residues indicates tat-III as a strong nucleic acid binding protein. Also, HIV-LTR sequences located 5'- to the transcription start site are known to be involved in trans-activation. Such upstream sequences would be important in generating a stable and open preinitiation complex with tat-III or induced non-viral factors or both. The combined entities could serve as an active launching pad for RNA polymerase II, enabling it to access the complex repeatedly.

SIGNIFICANCE

The present studies provide direct evidence for the involvement of the tat-III protein on the transcription of HIV genes. These in vitro transcription and gel retardation analyses will be extremely useful for the analyses of viral latency and the mechanisms involved in transcriptional activation. Thus, they can provide a strategy for the control of the virus infection and its propagation.

PROPOSED COURSE

1. As described above, these studies will be pursued to define the precise role of tat-III protein with the help of purified cellular components and tat-III protein, generation of specific antibodies against tat-III, and the development of a reconstituted transcription system.

2. In the analysis of latent, restricted and reproductive infections of the virus in monocytes, an attempt will be made to identify the negative/positive regulatory elements responsible for activation and expression of HIV genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09316-01 LBP

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cloning of Xenopus cDNA Encoding for Proteins Involved in Signal Transduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Kung	Chief	LBP, NCI
Others:	I. Calvert	Chemist	LBP, NCI
	K. Chen	IRTA	LBP, NCI

COOPERATING UNITS *(if any)*
BCDP, PRI, NCI-FCRF (Z. Peng)

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INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.0	1.0

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is accumulating evidence that phosphoionositol-specific phospholipase C (PI-PLC) is regulated by a G protein in a various biological system. PI-PLC is an important enzyme in phosphoinositide metabolism leading to the generation of two second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 can release Ca+2 from intracellular stores and DAG is an activator of protein kinase C (PKC). Xenopus oocytes have become an ideal system for the studies of signal transduction. To identify and characterize the proteins involved in signal transduction, we have isolated and characterized cDNA clones encoding Go-alpha, Gi-alpha, Gs-alpha, ADP-ribosylation factor (ARF), ras, phospholipase C, and two species of PKC from Xenopus oocytes cDNA library. Most of these cDNA clones have been fully sequenced.

Comparison of the sequences of the coding regions with mammalian cDNAs shows good homology at the nucleotide and deduced amino acid level. The conserved nature of amino acid sequences between Xenopus and mammalian species suggests that the proteins involved in signal transduction are important in evolutionary history. Northern blot analysis showed that mRNA of Xenopus Go-alpha was expressed more abundantly in brain tissue.

In addition, we have molecularly cloned the ARF cDNA from both human and Xenopus. The ARF is a 21 KDa GTP-binding protein that serves as the cofactor in the cholera toxin catalyzed activation of the regulatory subunit of adenylate cyclase (gs). There is also a high homology of ARF cDNAs between different species. The availability of these cDNA clones will make it possible to pursue structure function studies and the regulation of the expressions of these proteins.

PROJECT DESCRIPTION

PERSONNEL

Hsiang-fu Kung	Chief	LPB, NCI
Ida Calvert	Chemist	LBP, NCI
Kuang-Hua Chen	IRTA	LBP, NCI

INTRODUCTION

The G proteins, a family of guanine nucleotide-binding proteins, mediate numerous transmembrane hormonal and sensory transduction processes in eukaryotes. These proteins are essential for coupling the extracellular signals generated by activated membrane receptors to intracellular effector enzymes. There is accumulating evidence that phosphoionositol-specific phospholipase C (PI-PLC) is regulated by a G protein in a various biological system. PI-PLC is an important enzyme on phosphoinositide metabolism leading to the generation of two second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 can release Ca+2 from intracellular stores and DAG is an activator of protein kinase C (PKC). Xenopus oocytes have become an ideal system for the studies of signal transduction. To identify and characterize the proteins involved in signal transduction, we have isolated and characterized cDNA clones encoding Go-alpha, Gi-alpha, Gs-alpha, ADP-ribosylation factor (ARF), ras, phospholipase C, and two species of PKC from Xenopus oocytes cDNA library.

OBJECTIVES

The objectives of this project are to study the structure and function of the proteins involved in signal transduction and the regulation of their expressions.

METHODS

Molecular cloning, DNA sequencing, Southern and Northern blotting, nuclease S1 mapping and gene expression.

MAJOR FINDINGS

1. Xenopus Go-alpha, Gi alpha, ras, and two species of PKC cDNA's have been cloned and fully sequenced. Although the Go-alpha and Gi-alpha share considerable homology (about 60% homology), the conservation between species is far more dramatic. There is 85% homology at nucleotide level and 95% homology at amino acid level between Xenopus and mammalian species (human, bovine and rat). These results suggest that (1) most amino acid residues in G proteins may be important for their normal function in evolutionary history, and (2) the existence and conservation of these G protein families may reflect the functional requirement in desperate signal transduction system.

2. The message size and tissue distribution of the Go-alpha clone was determined by Northern analysis. The Go-alpha specific probe hybridized to two

messages, 5.0 and 2.0 Kb. The origin of the two Go-alpha messages is not known. Go-alpha is abundantly expressed in brain tissue. The significance of this differential expression between tissues is not clear, but may be related to the numbers and types of receptors and/or effectors that are expressed in each of the cell types of the tissue.

3. ARF is a novel GTP-binding protein with structural features of both the *ras* and G-protein families. In order to further determine the relationship between ARF and other members of the superfamily of GTP-binding regulatory proteins we have cloned and sequenced ARF from human and *Xenopus*. Comparison of human ARF sequence with bovine ARF shows 90.5% homology at the nucleotide level and 100% identity at the deduced amino acid level. It is very interesting that there is a common GC rich sequence (CCTTGGCCAGTG) at 5' non-encoding region in all G protein cDNAs.

4. Two species of *Xenopus* PKC cDNAs have been isolated and fully sequenced. The *Xenopus* PKC sequences share considerable homology with bovine and human PKC-II cDNAs. The sequence within the functional domains of PKC are almost identical among all PKC cDNAs.

FUTURE PLANS

To complete the characterization of 5' regulatory regions of Go-alpha, Gi-alpha, *ras*, ARF, and PKC genomic clones in order to understand the regulation of expressions of these proteins.

To complete the characterization of the cDNA clones encoding Gs-alpha and phospholipase C.

To determine the expression levels of mRNAs at various developmental stages in order to evaluate the possible roles of these proteins in *Xenopus* development.

To express these proteins in large quantities using our expression systems in order to pursue structure function studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09317-01 LBP

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Expression of Go-Alpha and Cytokines in Various Human Malignancies

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	J. Clark	Senior Staff Fellow	CRB, NCI
Others:	S. Lavu	Senior Staff Fellow	LBP, NCI
	H. Kung	Chief	LBP, NCI
	L. Helman	Chief	PB, NCI
	M. Israel	Senior Staff Fellow	PB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Physiology

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Go-alpha is a member of the family of guanine nucleotide binding (G) proteins which mediate signal transduction. Its function has not been fully elucidated. Different tissues have very different levels of Go-alpha protein with the highest levels being found in brain and neuroendocrine tissues. A preliminary study has shown high levels of Go-alpha in the serum from patients with a variety of neuroendocrine tumors but not in patients with other malignancies. We have cloned a human cDNA Go-alpha and have used this as a probe to evaluate the expression of Go-alpha mRNA in various malignancies by Northern blot analysis. Preliminary findings show increased levels of message in patients with malignancies of neuroendocrine origin (medullary thyroid carcinoma, pheochromocytoma, gastrinoma, glucagonoma and carcinoid) but not in other malignancies (e.g. squamous cell cancer of the lung, colon cancer and glioblastoma). Studies probing additional neuroendocrine and other malignancies both by Northern blot analysis and in situ hybridization, are ongoing in order to evaluate the value of Go-alpha as a marker for tumors of neuroendocrine origin. We hope to discover any possible role it might have in these malignancies.

We have also evaluated the expression of various cytokines at the mRNA level in two human melanoma cell lines, since several immunomodulatory activities have been detected in the supernatants of melanoma cell lines. These factors may play a role in modulating the immune response to melanoma. One of the cell lines (WM-9) constitutively expressed mRNA for IL-1 alpha, GMCSF, and PDGF, whereas the other (KRFM-2) expressed GMCSF and PDGF but not IL-1 alpha. Neither cell line had IL-1 beta mRNA. Thus, there is heterogeneity in the expression of various cytokine mRNAs in melanoma cell lines. Further evaluation of the expression of various cytokine mRNAs is progress.

PROJECT DESCRIPTION

PERSONNEL

Jeff Clark	Senior Staff Fellow	BTB, NCI
Sukadev Lavu	Senior Staff Fellow	LBP, NCI
Hsiang-fu Kung	Chief	LBP, NCI
Lee Helman	Chief	PB, NCI
Mark Israel	Senior Staff Fellow	PB, NCI

OBJECTIVES

Go-alpha is a member of the family of guanine nucleotide binding (G) proteins that mediate signal transduction from cell surface receptors to effector enzymes. Different levels of Go-alpha are found in different tissues. Particularly high levels are found in normal brain, other normal and malignant neuronal and neuroendocrine tissues, and endocrine cells in the adrenal, pituitary, thyroid, and pancreatic regions. The function of Go-alpha has not been elucidated. A preliminary study has shown high levels of Go-alpha in the serum from patients with a variety of neuroendocrine tumors, but not in patients with other malignancies. Based on these findings, we have examined Go-alpha mRNA expression in neuroendocrine and non-neuroendocrine tumor samples.

Since multiple immunomodulating cytokine activities have been detected in the supernatants of cultured human melanoma cell lines, we have also evaluated the mRNA expression of various cytokines in two of these cell lines. The production and modulation of these melanoma derived cytokines may play a role in the immune response to melanoma.

METHODS

Northern blot analysis, *in situ* hybridization.

MAJOR FINDINGS

Preliminary results show increased level of Go-alpha message in patients with malignancies of neuroendocrine origin (medullary thyroid carcinoma, pheochromocytoma, gastrinoma, glucagonoma and carcinoid) but not in other malignancies (e.g. squamous cell cancer of the lung, colon cancer, and glioblastoma).

We have shown that one of the melanoma cell lines (WM-9) constitutively expressed mRNA for IL-1-alpha, GMCSF, PDGF, whereas the other (KRM-2) expressed GMCSF and PDGF, but not IL-1-alpha. Neither expressed IL-1-beta mRNA. Thus, there is heterogeneity in the expression of various cytokines in melanoma cell lines.

FUTURE PLANS

Studies probing additional neuroendocrine and other malignancies both by Northern blot analyses and *in situ* hybridization, are ongoing in order to evaluate the value of Go-alpha as a marker for tumors of neuroendocrine origin. We hope to discover any possible role it might have in these malignancies. In addition, we are also analyzing the mRNA from various well characterized neuroblastoma cell lines in order to evaluate whether or not the level of Go-alpha mRNA expression correlates with other features of these cell lines.

Further evaluation of the expression of various cytokine mRNAs in melanoma samples is ongoing. These cytokines may play a role in modulating the immune response to melanoma.

PUBLICATIONS

Schultz AM, Tsai SC, Kung HF, Oroszlon S, Moss J, Vaughan M. Hydroxylamine-stable covalent linkage of myristic acid in Go-alpha, a guanine nucleotide-binding protein of bovine brain. Biochem Biophys Commun 1987;146:1234-239.

SUMMARY REPORT

CLINICAL RESEARCH BRANCH

October 1, 1987 to September 30, 1988

INTRODUCTION

The Clinical Research Branch (CRB) is responsible for the investigation of the therapeutic efficacy, toxicity, and mechanisms of action of biologicals and biological response modifying agents in patients with cancer. This branch of the Biological Response Modifiers Program was established to facilitate early clinical trials of biological products with potential as anticancer drugs. The unit is located at Frederick Memorial Hospital and was initially opened to the public in May 1981. The unit expanded over the past two years to a thirteen-bed inpatient unit that includes a four-bed monitoring unit for the treatment of patients receiving interleukin-2 plus lymphokine activated killer cells or high-dose interleukin-2 regimens. In addition, there is a 4-bed pheresis unit and a 10-bed outpatient unit/day hospital. This branch is particularly concerned with indepth Phase I and II trials of biologicals and BRMs involving small numbers of patients. Optimal immunomodulating doses as well as maximally tolerated doses of these drugs are being investigated for their therapeutic potential. In addition, a major goal of the branch is the integration of biological therapy with combination chemotherapy.

Over the past year the CRB has continued and expanded studies utilizing interleukin-2 with or without lymphokine activated killer cells. On our initial trial (see last year's summary), we treated 30 patients with a variety of refractory malignancies using a bolus IL-2 regimen of 100,000 units per kilogram intravenously every eight hours both for priming and for driving in vivo the in vitro generated LAK cells. Twenty-seven patients were evaluable for response. Two partial responses were observed. During the past year, we initiated a trial in which the priming schedule was changed to a continuous infusion regimen of 3 x 10^6 units/m^2 over 24 hours for five consecutive days. After a 36-hour rest, patients underwent leukapheresis for three consecutive days instead of five, as had been done previously. The yield of LAK cells on days 4 and 5 of our previous bolus regimen was markedly reduced compared to days 1 and 2 and thus leukapheresis was performed only on days 1-3. After the third leukapheresis, patients then received daily infusions of previously harvested LAK cells for three consecutive days along with the same interleukin-2 regimen at 3 million units/m^2 over 24 hours given for 5 consecutive days.

Although direct comparisons with our previous group of patients treated with the bolus regimen is difficult, the rebound lymphocytosis, number of LAK cells generated and number of LAK cells ultimately given back to the patients was significantly greater with the continuous infusion regimen. We have now treated 20 patients on this regimen and have observed only one partial response in a patient with renal cell carcinoma that is lasting now greater than four months. Thus, we have not seen an apparent increase in efficacy with the continuous infusion regimen despite our improvement in LAK cell yield. The toxicity of the continuous infusion regimen was less than that observed in the bolus IL-2 regimen.

We have continued our studies with interleukin-2 and LAK cells administered intraperitoneally. Twenty-three patients have now been treated, and the study is closed to further patient entry. Of these twenty-three, twenty-two are evaluable for response. There were five partial responders out of ten patients with colon carcinoma limited to the peritoneal space and two partial responders among nine evaluable patients with ovarian carcinoma. Because in this study LAK cells and interleukin-2 were administered intraperitoneally, we were able to monitor the degree of LAK cell activation and other immunologic parameters at the site of the tumor. We found that when LAK cells were administered intraperitoneally without interleukin-2, LAK cell activity rose but rapidly decreased back to baseline levels within approximately 8 hours. When interleukin-2 was administered with LAK cells however, there was a progressive increase in LAK activity over time that once again declined to baseline within approximately 48 hours after interleukin-2 was discontinued. The interleukin-2 dose we utilized in this study was 25,000 units per kilogram intraperitoneally every 8 hours. This dosing regimen resulted in peak ascites IL-2 levels of between 80,000 and 100,000 units of interleukin-2 per ml and trough levels of between 3,000 and 5,000 units of interleukin-2 per ml. These levels of interleukin-2 are certainly adequate to maintain LAK cell activity in vitro and are consistent with the observation that we were able to maintain LAK cell activity in vivo.

We evaluated serial ascites samples for the presence of secondary lymphokines and monokines produced in vivo during the course of administration of LAK cells and interleukin-2. IL-1 and TNF were not detected in the ascites fluid, but gamma interferon levels rose progressively over time to values peaking between 20 and 50 units per ml. Monocytes isolated from the peritoneal cavity showed progressive degrees of activation as reflected in their enhanced ability to produce hydrogen peroxide. What role, if any, these activated monocytes had in the antitumor effects we observed in this study is impossible to say, but our findings are consistent with a cascade effect of lymphokines in vivo.

We had hoped that by administering interleukin-2 intraperitoneally, we could limit systemic toxicity while maintaining LAK cell activation at the site of the tumor. LAK activity, in fact, was not found in the peripheral blood of these patients, and interleukin-2 levels in the serum were consistently less than 20 units/ml. Thus the desired pharmacologic advantage of intraperitoneal IL-2 administration had been realized. However, intraperitoneal fibrosis was a late and unexpected toxicity of this approach. Although the mechanism by which the fibrous tissue formed is not clear, it is clear that intraperitoneal fibrosis has limited our ability to give this type of therapy repetitively as had been hoped. Indeed, most partial responses in this study occurred after just one full month of therapy. If cycles of therapy could have been repeated many times, it is possible that a higher response rate and complete remissions could have been observed. Further studies will attempt to prevent the fibrosis. We intend to initiate a new study in which gamma interferon and colchicine will be administered to patients during the course of their treatment to limit the degree of fibrosis. Each of these agents has been demonstrated to decrease fibrous tissue formation in response to various stimuli, and it is hoped that they can inhibit fibrosis in vivo.

In addition to administering interleukin-2 with LAK cells, we have initiated a trial in which interleukin-2 is given alone in an attempt to determine if significant numbers of LAK cells can be generated in vivo and maintained at reasonable levels for prolonged periods of time. Obviously, this would not be possible in patients receiving doses of interleukin-2 used previously with LAK cell therapy as these doses are excessively toxic and can be administered for only short periods of time. The regimen we utilized consisted of an induction phase during which time patients would receive twice weekly doses of interleukin-2 at a dose of 3 x 10^7 per m^2 as a continuous IV infusion over 24 hours. Patients were monitored during the course of this treatment by serial determinations of LAK activity in the peripheral blood, for the production of secondary lymphokines, and for tumor responses. After the initial three weeks of induction therapy, if patients had achieved Leu-19 positive cell numbers greater than 2,500 per cubic millimeter, a lower dose maintenance phase of interleukin-2 was continued thereafter. Patients were evaluated at the three month time point for tumor response, and patients with stable tumors, minor responses, partial responses, or complete responses are continued on this dose of interleukin-2 indefinitely as long as Leu-19 levels remained above 20 percent of the peak Leu-19 level observed at the end of induction therapy. Low levels of LAK activity have been found in the peripheral blood of these patients, and when the total circulating LAK activity was calculated, it amounted to approximately one-third of the LAK activity that had been generated in vitro in our previous LAK plus IL-2 trials. Of course, we have no way to measure the size of the extravascular pool of LAK cells and, thus, cannot claim that the total body level of LAK activity was equal to, less than or greater than the amount of LAK activity that could be generated during the course of LAK and IL-2 therapy. Importantly, however, LAK activity could be maintained for as long as interleukin-2 was administered. Thus, one of the desired goals of the study, namely, in vivo induction of LAK activity and maintenance of LAK activity for prolonged periods of time, was achieved. Unfortunately, so far only four patients have had transient tumor regression lasting for less than one month and, thus, not qualifying as partial responses. Thus, it appears that if interleukin-2 is to be used alone as an antitumor agent, alternative means of enhancing its in vivo activity should be investigated.

Toward this end, we have initiated a trial combining interleukin-2 with flavone acetic acid (FAA). FAA has just completed phase I trials in a number of centers. Studies conducted in the laboratories of the Biological Response Modifiers Program have shown that when FAA is combined with interleukin-2, the combination has synergistic antitumor effects in a renal cell carcinoma model. In addition, very high levels of interferons can be detected in the serum of patients receiving FAA, and FAA administration can lead to a transient enhancement of natural killer cell activity in the peripheral blood. Some toxic effects of FAA, however, overlap with those of interleukin-2, and the protocol we are conducting is a phase I dose escalation trial. In addition to monitoring LAK cell generation in vivo, interferon levels and NK activation are being assayed. We have admitted five patients to this study and have observed no synergistic toxic effects and no antitumor effects so far. We will continue to dose escalate these agents in our attempts to mimic as closely as possible the promising laboratory results obtained previously. In addition, in the coming year, we plan to initiate a trial of interleukin-2 in combination with Poly ICLC another interferon inducer and another agent that has shown synergistic antitumor effects when given with interleukin 2.

While a great deal of attention has been paid to investigating antitumor effects of interleukin-2, we continue to perform studies aimed at achieving systemic activation of monocytes and macrophages with the ultimate hope that such activation might result in tumor regressions. Human monocytes can be activated to a tumoricidal state by a number of different mechanisms, and animal studies have suggested that doses of monocyte activating agents that are capable of optimally stimulating monocytes may be quite different and much lower than the maximally tolerated doses of these agents. To investigate this possibility in humans, we conducted a dose escalation trial of recombinant interferon gamma in patients with malignant melanoma with minimal tumor burden. Patients chosen for this study were clinically disease-free at the time the gamma interferon was administered so as to minimize any effects the tumor might have on gamma-interferon-induced macrophage and monocyte activation. Gamma interferon was given in doses spanning greater than four logs of drug dose, and using monocyte activation parameters that included monocyte hydrogen peroxide generation, Fc receptor expression and HLA-DR expression, it was found that, as in animals, the optimal immunomodulating dose of interferon gamma was substantially lower than the maximally tolerated dose. In fact, the optimal immunomodulating dose of gamma interferon in humans (which we determined to be 0.1 milligrams/m^2 intramuscularly daily or subcutaneously daily) was well tolerated with no patients receiving this dose requiring discontinuation of therapy for toxic effects. This is the first study which has demonstrated an optimal biologic dose of any biologic agent in humans. Having determined this optimal biologic dose, it is now important to follow up on this observation and determine if this dose has antitumor activity. The Southwest Oncology Group, in fact, is conducting a trial in which melanoma patients rendered disease-free by surgery are randomized either to no therapy or to treatment with the optimal biologic dose of interferon gamma. In addition, we have recently begun a study using various doses of interferon gamma in patients with advanced melanoma with measurable disease. Three dose levels of interferon gamma will be evaluated in this study for immunomodulating effects. The optimal immunomodulating dose may be different in patients bearing tumor than in the adjuvant setting. Once an OID is found, patients will be treated at this dose level to evaluate antitumor effects.

Other methods of augmenting monocyte and macrophage function have been investigated as well. We are conducting a phase I trial with muramyltripeptide administered intravenously in lipid vesicles. Patients are being monitored for toxic effects as well as immunomodulatory and antitumor effects. In addition, we have conducted a phase I trial of recombinant human GM-CSF in patients with solid tumors to evaluate not only the toxic and hematopoietic effects of this agent but also to determine its effects on the state of activation of monocytes and granulocytes. Although this agent is capable of increasing substantially the number of granulocytes and monocytes in the peripheral blood, we have been unable to convincingly demonstrate activation of these cells in vivo. However, one can hopefully combine recombinant human GM-CSF with more traditional monocyte activators such as gamma interferon (which is also capable of enhancing granulocyte function) in an attempt to bring about augmentation in effector cell number and function.

Obviously, an important component of the use of activated monocytes would be some knowledge of their ability to traffic to sites of tumors. We have been conducting a study using ^{111}Indium labeled activated and unactivated monocytes administered intravenously to patients with cancer to determine if activated cells are capable of homing to sites of disease. Patients underwent serial nuclear medicine scans following intravenous administration of these cells, and the pattern of distribution of these cells was similar whether or not they were activated with interferon gamma in vitro. Monocytes did not traffic to sites of tumor but distributed to liver, spleen and the intravascular compartment. This result would indicate that if one wished to use activated monocytes in the treatment of cancer, some mechanism of directing the monocytes to the site of disease would be necessary.

One way to circumvent the trafficking requirements for monocytes to the sites of the tumor would to administer the monocytes into an anatomic compartment in direct contact with the malignant disease. This is being attempted in a study using in vitro gamma interferon-activated monocytes administered into the peritoneal cavity of patients with colon carcinoma limited to the peritoneal space. A previous trial conducted at the BRMP showed that this approach was feasible in that large numbers of monocytes could be harvested and reinfused into the peritoneal cavity with acceptable toxicity. However, the study was performed on patients who had been rendered disease-free by an extensive surgical procedure prior to administering the monocytes intraperitoneally. Several of the patients on this trial have now gone for more than two years disease-free but it is unclear whether the monocytes are responsible. Our current study involves only patients with evaluable or measurable disease in the peritoneal cavity.

In an effort to activate monocytes in vivo, we have conducted a study of tumor necrosis factor with gamma interferon. In vitro and animal studies have demonstrated synergistic or additive antitumor effects of this combination of cytokines. We performed a phase I dose escalation study and determined the maximal tolerated dose as well as the optimal biologic dose. In contrast to what had been expected, increasing doses of tumor necrosis factor inhibited the gamma interferon activation of monocytes. If the combination of tumor necrosis factor and gamma interferon brings about tumor responses by activating monocytes in vivo, simultaneous administration of these agents would seem unlikely to produce the synergistic antitumor effects seen in vitro.

Over the past year the Clinical Research Branch has continued its studies in patients with hairy cell leukemia. The original study with interferon alfa-2a continues and in general patients continue to tolerate the therapy and to have stable responses to this treatment. However, the optimal method of maintaining response in patients with hairy cell leukemia has not been determined. Studies done elsewhere have shown that if interferon is discontinued after one or two years of therapy, patients will begin to relapse shortly thereafter. As most hairy cell leukemia patients treated with interferon do not achieve complete remissions, preventing disease progression is an important issue. It appears that those patients who restart therapy after progression of disease respond to second and third cycles of treatment with interferon. It is not clear whether one could maintain the response more effectively with chronic low dose therapy or intermittent maintenance cycles. Our study using continuous maintenance therapy will be compared to others using intermittent maintenance therapy.

We have analyzed the development of resistance to interferon. Thirty-one of fifty-one patients developed anti-interferon antibodies. Approximately half of these antibodies had the ability to neutralize interferon alfa-2a in vitro. Among the sixteen patients with neutralizing antibodies, six acquired resistance to interferon alfa-2a after having initially responded to this agent. Importantly, we did not observe acquired interferon resistance in patients with non-neutralizing antibodies or in patients without detectable anti-interferon antibodies. The reason why the remaining ten patients with neutralizing antibodies continued to respond to interferon is not clear; perhaps the response they achieved to interferon at the time they developed neutralizing antibodies is being maintained in the absence of the delivery of effective therapy. Such an explanation would be in keeping with the observed duration of response following discontinuation of interferon in other studies. To investigate further the biologic effects of anti-interferon antibodies in patients with hairy cell leukemia, we are conducting a study in which biologic endpoints (2' - 5' oligoadenylate synthetase activity in peripheral blood mononuclear cells, neopterin levels, interferon-induced fever, and interferon pharmacokinetics) are being measured following a standard dose of interferon alfa-2a in patients with and without antibodies. As the neutralizing antibodies in these patients did not have a measurable effect on the biologic effects in vitro of a partially purified preparation of natural interferon, we are also administering to these patients a standard dose of human lymphoblastoid interferon. If, as our hypothesis predicts, neutralizing anti-interferon antibodies uniformingly inhibit the in vivo effects of interferon alfa-2a in patients with hairy cell leukemia, and if biologic effects of human lymphoblastoid interferon are not inhibited in the face of these anti-interferon antibodies, human lymphoblastoid interferon might be an effective alternative therapy to patients becoming resistant to interferon alfa-2a in association with antibodies. This would be an important conclusion to reach as alternative therapies for interferon-resistant patients at the moment include only deoxycoformycin, a drug we have recently found to be a potent immunosuppressive agent.

Because we have found deoxycoformycin to be an effective salvage therapy for patients resistant to interferon alfa-2a, we initiated a trial combining deoxycoformycin and interferon alfa-2a in this disease. Thirteen patients are evaluable for response and all thirteen have had clearing of all but a very small percentage of hairy cells from the bone marrow. Patients normalized their peripheral blood counts quickly and the toxicity of this approach was acceptable. We evaluated the bone marrow of these patients serially with bilateral iliac crest bone marrow biopsies performed a minimum of two months apart. The marrow generally is the last site of disease to respond and thus complete responses could only fairly be judged by a more thorough evaluation of marrow than had been reported in previous studies where only unilateral iliac crest marrow biopsies were performed once to assess response. Because all thirteen evaluable patients in our study combining dCF with interferon alfa had small numbers of residual hairy cells in the marrow, we had no complete remissions on this study. However, we believe these results, in contrast to other studies with deoxycoformycin alone in which high percentages of complete remissions were reported, indicate that treatments with deoxycoformycin with or without other agents are incapable of giving high proportions of complete responders if one were to adequately evaluate the marrow following therapy. Recently, patients treated with deoxycoformycin elsewhere and evaluated for response by more relaxed criteria are beginning to relapse from their "complete remissions" consistent with our observation of persisting disease in our patients. Whether therapy combining interferon alfa-2a with deoxycoformycin is superior to deoxycoformycin alone can only be determined by comparing long term response durations.

Because deoxycoformycin is an inhibitor of adenosine deaminase and this enzyme is deficient in about 1/2 the cases of severe combined immunodeficiency disease, we evaluated immune function in patients receiving combination interferon alfa-2a and deoxycoformycin. Not surprisingly, T-cell numbers, including both CD4 and CD8 T-cell subsets, were markedly decreased early in the course of therapy. Total CD4 cell numbers fell to less than 200 per cubic millimeter in all patients evaluated. This drop in CD4 positive cell numbers was associated with marked decreases in the proliferative responses of peripheral blood mononuclear cells in vitro to a number of lectins. Patients have now been evaluated for up to eight months following completion of all therapy. Immune suppression persists to a significant degree in most patients although there appears to be some recovery of lectin-induced proliferative responses.

The clinical consequences of this immune suppression remain to be fully evaluated. We have not observed opportunistic infections in any of our patients to date. However, four of the fifteen patients on this study have developed localized Herpes zoster late in the course of therapy or after discontinuation of therapy. In three of the cases, patients were treated early with acyclovir and dissemination did not occur. In the fourth case, acyclovir was not administered and dissemination did not occur. One patient with hairy cell leukemia from another center treated with deoxycoformycin developed Pneumocystis carinii pneumonia. Immune function in this patient at the time of her PCP was markedly suppressed and the total CD4 cell number in the peripheral blood was only 38 per cubic millimeter. She was found to be anti-HIV negative. Thus, the potential for important clinical consequences of this immune suppression exists and we continue to follow our patients closely for any clinical manifestations.

The Clinical Research Branch has completed a trial of human monoclonal antibodies in patients with colorectal carcinoma in the past year. In previous studies murine monoclonal antibodies have been found to be capable of localizing to sites of tumor, to rarely bring about antitumor effects, but to routinely elicit an antimouse antibody response. This response is an important limiting factor in the chronic use of monoclonal antibodies in patients with cancer. The development of human monoclonal antibodies raised the possibility that one might circumvent the development of human antimouse antibody responses. Two such antibodies were recently developed and showed reactivity with colon carcinoma cells. We have investigated their ability to localize to sites of disease in patients with disseminated colon cancer following labeling of the antibodies with ^{131}I. These antibodies gave tumor scans that were comparable to those obtained with murine monoclonal antibodies: only large (>2 cm) masses were visualized. Importantly, these antibodies were not immunogenic in man. No patient developed antibodies to the human monoclonal antibody during the course of this study. The stage is now set, therefore, for coupling this antibody to therapeutic amounts of radioactive materials for use as antitumor agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09278-05 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biodistribution and Trafficking of Normal and Activated Blood Monocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Smith II	Senior Staff Fellow	CRB, NCI
Others:	J. Beman	Nurse Specialist	CRB, NCI
	P. Miller	Biologist	OAD, NCI
	R. G. Steis	Chief	CRB, NCI

COOPERATING UNITS *(if any)*

Nuclear Medicine, Clinical Center, NIH (A. Keenan); Frederick Memorial Hospital, Frederick, MD (M. Farrell); Genentech, San Francisco, CA (S. Sherwin)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research study is designed to assess the trafficking pattern of autologous monocytes when given intravensously to humans. Monocytes will be removed from normal volunteers or from cancer patients by cytapheresis and purified by counter-current centrifugal elutriation. The purified monocytes will either be left at 4 degrees (normal monocytes) for 4 hours or be activated by exposure to recombinant human gamma interferon. The cells will be labeled with Indium-111 and infused intravenously back into the normal donor or patient, respectively. Normal donors or cancer patients will first be given an intravenous infusion of autologous normal monocytes; if no untoward reactions are noted, the donors or patients may receive infusions of autologous gamma interferon-activated monocytes four weeks later. Frequent blood samples will be withdrawn from the patient or donor over the ensuing 96 hours to determine the rate at which the infused cells equilibrate with the marginating pool of blood monocytes (if one exists), and to determine the rate at which these cells emigrate from the bloodstream to enter the reticuloendothelial system and other tissues. In addition, multiple regional scintigraphs will be obtained to determine if these labeled monocytes preferentially localize to any one organ system. This information will further our understanding of the normal physiology of activated and unactivated monocytes and, in addition, hopefully will allow us to more rationally design adoptive immunotherapy trials in which purified autologous cytotoxic blood monocytes will be given to cancer patients intravenously.

PROJECT DESCRIPTION

PERSONNEL

John W. Smith II	Senior Staff Fellow	CRB, NCI
JoAnn Beman	Nurse Specialist	CRB, NCI
Paul Miller	Biologist	OAD, NCI
Ronald G. Steis	Chief	CRB, NCI

OBJECTIVES

The objectives of this study are:

1. To determine the toxicity in cancer patients and normal donors of intravenously infused autologous monocytes.

2. To study the biodistribution and trafficking of intravenously infused autologous monocytes.

3. To evaluate the possible antitumor effects of autologous monocytes in cancer patients.

METHODS EMPLOYED

Normal volunteers or patients will be admitted to the Biological Response Modifiers Program Cytapheresis Unit for leukapheresis. There will be minimal anticoagulation of the normal donor's or patient's blood using acid citrate dextrose and heparin. White cells will be collected for exactly 2 hours at which time approximately 5 x 10^9 mononuclear cells will have been collected using a Celltrifuge 2 cell separator or CS 3000 cell separator. The donors will then be admitted to the Biological Response Modifiers Program inpatient unit. The leukocytes collected by leukapheresis will then be subdivided into mononuclear cells using hypaque-ficoll gradients according to standard procedures. These leukocytes will then be washed 3X in RPMI 1640 media (sterile and endotoxin-free) and entered into an elutriator centrifuge in medium containing RPMI 1640 and clinical grade human albumin (Cutter Laboratories). The mononuclear leukocytes will be entered into the elutriator in a laminar flow hood, and the monocytes will also be collected out of the elutriator centrifuge in a similar hood. The purified monocytes will then be incubated at 4 degrees for 4 hours or exposed to .5 mcg/ml of recombinant gamma interferon for 4 hours at 37 degrees. Following the 4-hour incubation period, the cells will be washed twice in RPMI 1640, and 5 x 10^8 monocytes will be labeled with 0.25 millicuries of ^{111}indium oxine, according to the technique of Lotze et al. previously employed in our earlier EVLA studies. The ^{111}indium labeled monocytes will be washed twice in RPMI 1640 and resuspended in phosphate buffered saline + 10 percent albumin and infused into the patient or normal donor intravenously over

5 minutes. The total body maximum radiation dosage estimate for the ^{111}indium labeled monocytes administered intravenously has been calculated to be .710 rads/millicurie. Since only 0.1 of a millicurie will be injected per patient or normal donor, a maximum radiation exposure estimate per procedure is .071 rads of total body exposure. The critical organ is expected to be rads per study. Infused cells (in the medium in which they are suspended) will be routinely tested for endotoxins, bacteria and fungi; in addition, their purity, viability, and tumoricidal function will be monitored.

In order to determine the biodistribution of ^{111}indium labeled monocytes infused intravenously, whole body images and multiple regional scintiphotos of the torso will be obtained on a daily basis throughout the five days of the study and acquired on computer for later analysis. In addition, blood samples will be obtained every 10 minutes for the first 2 hours of the study, on an hourly basis for the next 16 hours of this study, and on a every 12-hour basis for the duration of the study.

MAJOR FINDINGS

Five cancer patients have completed treatment with this study using both activated and nonactivated autologous monocytes. No toxicity was seen with the nonactivated monocytes. Two patients receiving activated monocytes had minor toxicity (low grade fever in one; fever, fatigue, chills, and myalgias in the other) which was easily controlled symptomatically and resolved promptly by the end of therapy. Images obtained beginning 12 hours after injection showed trafficking of monocytes of liver, spleen, and bone marrow but not to known tumor sites. No antitumor responses were seen in any patient. Five normal donors have now received nonactivated autologous monocytes labeled with ^{111}indium. No toxicity was observed. In both the cancer patients and in normal donors, most of the monocytes remained in the peripheral circulation for a very short period of time. The number of counts per milliliter in the peripheral blood fell dramatically, rose slowly, and then declined slowly. This presumably reflects trafficking of these monocytes first to the lungs then to the extravascular spaces. The question of trafficking to the lungs remains speculation because the earliest images obtained were at 12 hours after injection. Images in normal donors revealed trafficking of monocytes to the liver, spleen, and bone marrow.

SIGNIFICANCE

For the last five years, several investigators in the BRMP have been characterizing the ability of highly purified human monocytes to kill cancer cells in vitro. It is now possible to routinely isolate up to 10^9 highly purified human monocytes from normal volunteers or cancer patients by the techniques outlined above and monitor their ability to kill cancer targets in vitro. Baseline cytotoxic capabilities of these monocytes can be routinely boosted by in vitro incubation with gamma interferon. The question that is being addressed in this particular protocol centers around the general applicability of adoptively transferred cytotoxic monocytes in the treatment of cancer. Since most patients with metastatic cancer do not have disease limited to one anatomic space, it would be most reasonable to attempt to approach these patients with adoptively transferred monocytes through intravenous infusions.

It appears from the preliminary results of the current study that either activated or unactivated monocytes do not home to sites of tumor. Subsequent studies might take advantage of this observation and try to target monocytes by incubating them not only with gamma interferon but also with appropriate monoclonal antibodies. One goal of the present study is to determine the biodistribution of activated and unactivated monocytes to determine if there is a need for alternative methods to attempt to make them home to sites of disease. It is felt that the present study is warranted because of human and animal in vitro data suggesting that monocytes when properly activated can have direct tumoricidal activity in vitro and because of animal in vivo experiments suggesting that activated macrophages can check the spread of or eliminate established tumors. The patient population in normal donors described in this protocol are reasonable for testing the trafficking of intravenously infused monocytes since there is no direct information as to the behavior of these cells (either in their baseline or activated stage) when infused intravenously. If a mechanism can be found whereby activated monocytes can be targeted to tumor sites, protocols will be developed for the treatment of widely disseminated metastatic cancer with such activated cells.

PROPOSED COURSE

This study has been closed.

PUBLICATIONS

Stevenson HC, Miller PJ, Alvord G, Keenan A, Larson S, Larcena L, Smith J, Longo DL. Trafficking and biodistribution of autologous peripheral blood moncytes in normal donors and cancer patients after intravenous administration. In: Journal of Leukocoyte Biology. Kauai 1987;42:(Abst. 79)355.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09291-03 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alternating 2'-dCF in Recombinant Leukocyte A IFN in Hairy Cell Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
Others:	A. Martin	Staff Fellow	DEB, NCI
	S. Nerenstone	Staff Fellow	DEB, NCI
	M. J. Hawkins	Head	BES, CTEP, NCI
	R. Simon	Chief	BR, NCI
	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (W. J. Urba)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because we have previously observed substantial responses to deoxycoformycin in hairy cell leukemia after failure of interferon, we began this pilot protocol to evaluate the efficacy and toxicity of combined recombinant interferon alfa-2a and 2'deoxycoformycin. Response criteria in this protocol were more strict than other studies in that we required absence of hairy cells from bilateral iliac crest bone marrow biopsies obtained twice at approximately two month intervals and improvement of the peripheral blood hemoglobin to greater than 12 grams percent, granulocyte count to greater than 1500 per cubic millimeter, and platelet count to greater than 100,000 per cubic millimeter. Most previous studies using deoxycoformycin have declared patients complete responders on the basis of a single unilateral bone marrow biopsy. Of 15 patients, 13 are evaluable for response with one patient being inevaluable because of a diffuse bony sclerosis and consequent inability to provide marrow for assessment of the marrow response, and one patient continues on therapy. All 13 patients had partial responses with small numbers of residual hairy cells remaining in the bone marrow. Had standard criteria for complete remission been used, we would have had 10 complete responses based on only a single unilateral bone marrow biopsy. It appears that the combination of deoxycoformycin plus interferon provides no substantial improvement in complete response rate over dCF alone. At present, therefore, the combination of deoxycoformycin and recombinant interferon alfa-2a cannot be recommended over either agent alone.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Allison Martin	Staff Fellow	DEB, NCI
Stacy Nerenstone	Staff Fellow	CTEP, NCI
Richard Simon	Chief	BR, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

1. To determine the qualitative and quantitative toxicities of 2'-deoxycoformycin and interferon alfa given in alternating monthly cycles to patients with progressive hairy cell leukemia.

2. To gather preliminary data on the efficacy of this treatment combination by response rate and remission duration.

3. To determine the alternations in immune function as a result of treatment with alternating dCF and alfa interferon.

4. To determine the biochemical consequences of administering dCF with a biological response modifier.

METHODS EMPLOYED

Patients to be considered eligible for this study must have morphologically identifiable hairy cells in the peripheral blood, bone marrow or tissue biopsies including a positive stain for tartrate-resistant acid phosphatase. They must meet minimal criteria for peripheral blood counts prior to starting therapy. Hemoglobin must be less than 10 grams percent or patients must be transfusion dependent and/or the platelet count must be less than 100,000 and/or the absolute granulocyte count must be less than 1,500. Patients must have a performance status of greater than 70 percent and have an expected survival greater than three months prior to study entry. Prior therapy is allowed for patients entering this study, but no patients must have received prior alfa interferon or deoxycoformycin. Patients are evaluated in the outpatient clinic of the Clinical Research Branch and then are hydrated and given 4 mg/m^2 of deoxycoformycin intravenously as a one-half hour infusion. After infusion of the dCF, a further liter of fluid is administered to insure adequate hydration. Patients are serially evaluated for myelosuppression and receive weekly injections at this dose of deoxycoformycin for three consecutive weeks. On week 4 of the study no therapy is given, and in weeks 5 through 8 daily subcutaneous injections of recombinant alfa interferon at a dose of 3 million units/m^2 is administered. This two-month cycle of deoxycoformycin alternating with interferon is repeated for a minimum of 7 cycles. Initial response is evaluated by serial evaluations of peripheral blood counts and by bilateral iliac crest bone marrow biopsies performed at the 6-month time point. Bilateral iliac crest bone marrow biopsies are then obtained every two months thereafter and every six months after the completion of therapy. For patients clearly responding well to therapy and tolerating the therapy reasonably well, some of the deoxycoformycin injections are permitted to be administered by the local physician. These local physicians, however, must agree to follow the protocol exactly as written.

MAJOR FINDINGS

As mentioned, 15 patients have been admitted to this trial, and 13 are evaluable for response. One patient is still receiving therapy and another patient is not evaluable for marrow response because of diffuse bony sclerosis. All evaluable patients had a partial response with a reduction in the hairy cell infiltrate in the bone marrow to less than 5 percent of the infiltrate that was present initially and improvement in peripheral blood parameters to the levels mentioned previously. Had the more usual criteria of complete response been used in our study, 10 patients would have had a CR. However, with our requirement for bilateral bone marrow biopsies done twice a minimum of two months apart, no patient was a complete responder. The duration of response after completion of therapy remains to be determined. Only by determining the duration of response will it be possible to compare the relative efficacy of this approach to that obtained with deoxycoformycin alone.

The toxicity experienced in this protocol consisted of nausea and vomiting in thirteen patients, depression in eight, and paresthesias in five, dermatitis in ten, minor eye irritation without corresponding clinical signs in six and herpes zoster infections in four. The herpes zoster infections occurred late and may be indicative of an underlying suppression of immune function (see below). Hematologic toxicity included progressive neutropenia in all 15 treated patients early in the course of therapy, anemia requiring transfusions in three patients, and fever requiring admission to the hospital for antibiotics in five patients. Ten patients had less than 500 granulocytes/mm^3 early in the course of therapy, but most of these patients had granulocyte counts less than 500 prior to beginning deoxycoformycin.

Because deoxycoformycin is an inhibitor of adenosine deaminase and deficiency of this enzyme is associated with some types of congenital immunodeficiencies, we evaluated immune function in these patients serially and compared their immune function to patients receiving only interferon alfa. Patients receiving deoxycoformycin had rapid and very marked drops in total lymphocyte counts and in total numbers of CD4 and CD8 positive lymphocytes. Although most patients had normal CD4 positive levels and normal T4 to T8 ratios prior to beginning therapy, all patients had decreases in CD4 positive lymphocyte numbers to less than 200/mm^3 shortly after the first course of deoxycoformycin therapy. CD4 positive cell numbers remained depressed for the duration of therapy and remain depressed for up to six months after completion of dCF. The duration of suppression of CD4 positive cell counts is being followed closely in these patients. As mentioned above, four patients had herpes zoster infections late in the course of therapy or after completion of therapy on this study. Three were treated with Acyclovir early in the course of therapy, and it is impossible to know whether they would have had disseminated disease had this therapy not been given. The fourth patient was not treated with Acyclovir and only had localized dermatomal disease. No patients in our study have had opportunistic infections to date.

SIGNIFICANCE

This study was undertaken primarily to improve the long-term response duration and complete remission rate of patients with hairy cell leukemia. Our definition of complete remission is different from that used in other studies in which deoxycoformycin is administered. Nonetheless, it does not appear that the combination of alfa interferon and deoxycoformycin will lead to greater degrees of initial response than that obtained with deoxycoformycin alone. However, only by comparison of the duration of response in our patients to the duration of response in patients receiving deoxycoformycin alone (and graded for response with more relaxed criteria) will it be possible for us to determine if this therapy is better, the same, or not as good as deoxycoformycin alone. Clearly, the regimen is reasonably well tolerated and brings about peripheral blood and marrow responses much more rapidly than with interferon alfa alone.

An additional point of interest in this study is that when one looks carefully in the bone marrow for residual disease, it can almost uniformly be found. Thus, other studies in hairy cell leukemia patients in which deoxycoformycin is being used need now to be reevaluated by more extensive testing of the bone marrow as was done in our study to determine if true complete response had occurred or if the apparent high complete remission rate in previous studies was merely related to sampling error of the bone marrow.

In addition, the observation of profound immunosuppression in our patients and the very prolonged duration of immune suppression has not previously been documented in patients receiving deoxycoformycin. Clearly, this aspect of this therapy will have to be evaluated very closely in our study and should also be done in other studies in which deoxycoformycin is being used. The immunosuppression observed following dCF might also be useful in diseases with an immunologic pathogenesis and for the manipulation of immune function in patients receiving various biologics.

PROPOSED COURSE

This trial has been closed to further patient entry but remains open for follow-up of patients previously treated.

PUBLICATIONS

Nerenstone S, Martin A, Urba W, Lawrence J, Clark J, Smith J, Crum E, Miller R, Schoenberger C, Hawkins M, Longo D, Steis R. Treatment of hairy cell leukemia (HCL) with alternating cycles of 2' deoxycoformycin (DCF) and recombinant alpha-2a interferon (IFN). In: Proceedings of the American Society of Clinical Oncology. New Orleans 1988;7:(Abst. 679)176.

Urba WJ, Smith JW, Longo DL, Baseler MW, Kopp WC, Clark JW, Steis RG. Deoxycoformycin (dCF) causes profound T cell dysfunction in patients with hairy cell leukemia. In: Proceedings of the American Society of Clinical Oncology. New Orleans 1988;7:(Abst. 642)166.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09292-03 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Adoptive Immunotherapy Utilizing LAK Cells & IL-2 Administered Intraperitoneally

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
	R. Ozols	Head	ETS, COP, NCI
Others:	J. Ortaldo	Chief	LEI, NCI
	D. L. Longo	Associate Director	OAD, NCI
	M. T. Lotze	Investigator	COP, NCI
	S. A. Rosenberg	Chief	SB, NCI
	M. Bookman	Senior Staff Fellow	MB, NCI

(See next page for continuation)

COOPERATING UNITS *(if any)*

NM, CC, Bethesda, MD (J. Carrasquillo, A. Keenan, S. Larson); DTM, CC, Bethesda, MD (E. Reed); Program Resources, Inc., Frederick, MD (W.Urba); Frederick Memorial Hospital, Frederick, MD (C. Schoenberger)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We sought to improve the efficacy of LAK + IL-2 therapy by administering it intraperitoneally to patients with cancer limited to this anatomic space. Patients underwent priming with interleukin-2 and harvesting of LAK precursor cells as is done on the systemic LAK and IL-2 trials. The cells were administered intraperitoneally daily for five days along with interleukin-2 at a dose of 25,000 units per kilograms every eight hours. Of ten evaluable colon carcinoma patients, five had partial responses, and of nine patients with ovarian carcinoma, two had partial responses. All responses were judged either by peritoneoscopy or by laparotomy and tended to be of short duration (four months). Toxic effects of this therapy were similar to those observed in patients receiving LAK and IL-2 therapy systemically with the exception that our patients routinely developed diffuse abdominal swelling and rebound abdominal tenderness. LAK activity was not maintained in the peritoneal cavity unless interleukin-2 was administered concurrently. LAK activity was observed in the peritoneal cavity for a minimum of five consecutive days while IL-2 was being administered. Activity fell back to baseline approximately 48 hours after discontinuation of intraperitoneal infusions of IL-2. Gamma interferon levels rose appreciably in the peritoneal fluid and there was evidence from monocyte activation in vivo. Intraperitoneal fibrosis limited the delivery of therapy beyond approximately three cycles. We conclude that this therapy is effective in bringing about tumor responses and is reasonably well tolerated. However, fibrosis in the peritoneal cavity will ultimately prevent the effective delivery of this therapy.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Robert Ozols	Head	ETS, COP, NCI
John Ortaldo	Chief	LEI, NCI
Dan L. Longo	Associate Director	OAD, NCI
Michael T. Lotze	Investigator	COP, NCI
Stephen A. Rosenberg	Chief	SB, NCI
Michael Bookman	Senior Staff Fellow	MB, NCI
Ami Ostega	Research Nurse	MB, NCI
Robert Young	Chief	MB, NCI

OBJECTIVES

1. To determine the toxicity of intraperitoneally administered LAK cells alone and of LAK cells plus interleukin-2.

2. To determine the antitumor activity of intraperitoneally administered LAK cells plus IL-2 in patients with epithelial ovarian cancer limited to the peritoneal cavity and peritoneal carcinomatosis arising from colorectal cancer.

3. To determine the in vivo trafficking of ^{111}In labeled IL-2 activated peripheral blood mononuclear cells by external scanning and by serial measurement of LAK cell activity in peripheral blood mononuclear cells after intraperitoneal administration of LAK cells.

4. To determine the pharmacokinetics of interleukin-2 in serum and peritoneal fluid after intraperitoneal administration.

5. To determine the biologic effects on peripheral blood mononuclear cells of intraperitoneally administered IL-2.

METHODS EMPLOYED

Any patient with disease outside of the peritoneal cavity, for example lung, liver, or retroperitoneal nodal metastasis is excluded from study. Patients subsequently undergo peritoneoscopy at either the Clinical Center or Frederick Memorial Hospital and a Tenckhoff catheter is placed. During placement of the Tenckhoff catheter, the abdomen is staged for the amount of disease and for the presence of malignant cells in washings or ascites.

Patients are primed with 100,000 units per kilogram of recombinant interleukin-2 intravenously every 8 hours for three consecutive days. Following two days of rest, the patients undergo a daily leukapheresis for 5 consecutive days. The cells thus obtained are transported to the LAK laboratory where they are purified by centrifugation over a cushion of Ficoll-Hypaque, and they are then incubated with interleukin-2 to generate LAK cells. After seven days of incubation with the interleukin-2, the cells are washed and resuspended in interleukin-2 containing saline solution. The cells are transported to the bedside and are administered intraperitoneally. Interleukin-2 is administered along with the LAK cells intraperitoneally at a dose of 25,000 units per kilogram every 8 hours. Patients receive intraperitoneal infusions of LAK cells and interleukin-2 for five consecutive days (5 total infusions of LAK cells and 15 total doses of interleukin-2) and the following 2 weeks undergo pheresis and infusions of LAK cells and interleukin-2 as outlined previously. Patients are evaluated for response, thus, after four and a half weeks of therapy total. Responding patients have returned for repeat administrations of LAK cells and IL-2.

MAJOR FINDINGS

If one assumes that LAK cells may mediate tumor cells lysis directly, then the effectiveness of this therapy might be enhanced by administering these cells directly into an anatomic compartment in direct association with the tumor. This was the basic rationale underlying the study, but in addition by administering the cells into an anatomic compartment that we could sample repetitively, we hoped to determine the in vivo state of activation of the cells and the IL-2 concentration in with which the tumor and the LAK cells would be in contact. If no tumor responses occurred in the face of adequate LAK activity, we could conclude that lack of efficacy in this situation would be related to resistance of the tumor cells to LAK-mediated lysis and not to inability of the LAK cells to traffic to sites of tumor.

Twenty-three patients were entered onto this study of which 23 are evaluable for toxicity and 22 for response. One patient developed a bowel perforation two days after beginning priming with interleukin-2 and was never treated with intraperitoneally administered LAK cells and interleukin-2. All patients had disease limited to the abdominal cavity and included nine patients with ovarian carcinoma, twelve with colorectal carcinoma, one with endometrial carcinoma, and one with a small bowel adenocarcinoma. Eleven patients with colon carcinoma are evaluable for response, and there were five partial responses. Nine patients with ovarian carcinoma were evaluable for response, and there were two partial responses. Both the patient with the small bowel adenocarcinoma and with endometrial carcinoma did not respond to therapy.

All responses in this study were assessed by repeat laparotomy or repeat peritoneoscopy. In two responding patients (one with ovarian carcinoma and one with colon carcinoma) only microscopic residual disease was present after intraperitoneal LAK and IL-2 therapy. All other patients had a substantial reduction in tumor volume in the peritoneal cavity but had gross residual disease at their second-look evaluation. The median response duration was short, four months. The toxicity of this therapy was similar to that observed in patients receiving systemic LAK and IL-2 and consisted of fluid retention with weight gain, oliguria, hypotension requiring pressors, fever and chills, nausea and vomiting, diarrhea, dyspnea, atrial fibrillation, and frequent premature ventricular contractions. Twelve patients developed dyspnea related to elevated hemidiaphragms; in no case did we observe diffuse or localized pulmonary infiltrates, and in no case was intubation required. Additional toxicities consisted of rash, confusion and disorientation, Tenckhoff catheter malfunction requiring replacement and bacteremia related to central venous catheters. One patient had a grand mal seizure which could not be attributed to either metabolic or structural causes and must be attributed to interleukin-2 administration. Four patients suffered a bowel perforation during the course of this study: one perforation occurred during replacement of a Tenckhoff catheter, one during priming with interleukin-2 (this perforation was at a sight distant from known areas of metastatic disease in the wall of the colon), one small bowel perforation occurred at the sight of insertion of the Tenckhoff catheter where the catheter came in contact with the small bowel, and the last perforation occurred at an unknown sight and could not be found at exploratory laparotomy. This last patient did not have recurrence of perforation after exploratory surgery. Of the twenty-three patients, there were six episodes of bacterial peritonitis and two of fungal peritonitis. In all cases except for one with fungal peritonitis, there were no associated abdominal findings; the peritonitis was picked up by routine screening cultures of the peritoneal fluid.

Perhaps the most important toxic effect of this therapy has been the development of intraperitoneal fibrosis. This fibrosis usually presented as a large pseudocyst in the abdominal cavity that communicated with the insertion site of the Tenckhoff catheter. When contrast material was instilled into the Tenckhoff catheter, the dye remained confined to the cavity of the pseudocyst; no contrast material was able to reach the rest of the abdominal cavity. Because our consulting surgeons felt that an extensive surgical procedure would be required to remove the pseudocyst in order to deliver further therapy (and there was no guarantee that after this extensive surgery that reformation of adhesions would not preclude further administration of therapy), this complication of therapy appears to limit our ability to deliver this therapy even in the face of documented effectiveness in some patients.

Immunologic monitoring of these patients was performed during the course of the treatment of these patients. Interleukin-2 levels in the peritoneal cavity reached a peak of between 80,000 and 100,000 units/ml, and troughs of approximately 3,000 to 5,000 units/ml. Concurrent serum levels of interleukin-2 rarely exceeded 20 units/ml. Thus, it appeared that we had attained the desired pharmacologic advantage of having high interleukin-2 levels at the site where LAK cells must be maintained in an active lytic state yet low systemic levels such that reduced systemic toxicity might result. Gamma interferon levels in the peritoneal fluid rose progressively during the course of the treatment to levels of between 20 and 50 units/ml. Concurrent with elevations in peritoneal gamma interferon levels there was activation of resident macrophages as assessed by their enhanced production over time of hydrogen peroxide. Interleukin-1 and tumor necrosis factor were not found in the ascites samples.

SIGNIFICANCE

Interleukin-2 and LAK cells administered as outlined in this study are capable of bringing about tumor responses in patients with disease limited to the peritoneal cavity. These tumor responses were associated with moderate degrees of toxicity but also with the late development of intraperitoneal fibrosis which limited the delivery of therapy to these patients. An important finding in this study not previously reported is that LAK cells in vivo can be maintained in an active lytic state when interleukin-2 is administered; such determinations were not possible in patients receiving intravenously administered LAK cells and IL-2 since LAK cells do not circulate when IL-2 is administered concurrently. In addition, we have demonstrated in this study the ability of secondarily induced lymphokines to be produced in these patients and that these secondary lymphokines can bring about activation of different cell populations that might be involved in the tumor responses observed.

PROPOSED COURSE

This study is now closed for further patient entry. Because there is therapeutic activity with this approach that is limited by the development of intraperitoneal fibrosis, if this therapy is to be used in the future, it should be coupled with some attempt to bring about a reduction in the formation of fibrosis in vivo. Gamma interferon has been shown to significantly inhibit the transcription of messenger RNA for types 1 and 3 collagen in normal dermal fibroblasts and in dermal fibroblasts from patients with scleroderma. It is currently undergoing a phase II trial in patients with scleroderma to determine if the skin fibrosis in this disease can be reversed. The concentrations of gamma interferon that bring about this effect are achievable with minimal toxic effects in the peritoneal cavity of patients with ovarian cancer. In addition, colchicine has been shown in a number of animal models to decrease fibrosis at sites of surgical incisions and to decrease the amount of fibrosis in experimental models of cirrhosis of the liver. In a revised version of this protocol, we intend to add pretreatments with gamma interferon and colchicine to the interleukin-2 and LAK cell regimen in an attempt to decrease the formation of fibrosis and to hopefully allow a more prolonged course of therapy in patients with these types of malignancies.

PUBLICATIONS

Steis R, Bookman M, Clark J, Urba W, McKnight J, Smith J, Schoenberger C, Ozols R, Young R, Maluish A, Beckner S, Longo D. Intraperitoneal lymphokine activated killer (LAK) cell and interleukin-2 (IL-2) therapy for peritoneal carcinomatosis: toxicity, efficacy, and laboratory results. In: Proceedings of the American Society of Clinical Oncology. Atlanta 1987;6:(Abst. 984)250.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09294-03 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Relapsed T Cell Lymphomas with Recombinant Leukocyte A Interferon

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
CoPI:	D. Ihde	Senior Investigator	NMOB, NCI
Others:	J. D. Minna	Chief	NMOB, NCI
	E. Sausville	Senior Investigator	NMOB, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith II	Senior Staff Fellow	CRB, NCI
	R. L. Miller	Senior Staff Fellow	CRB, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (W. Urba)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous trials have demonstrated significant antitumor activity of interferon alfa in patients with cutaneous T-cell lymphomas. The dose used in the original trial was the maximally tolerated dose of 50 million units per meter squared given intramuscularly three times per week. The response rate in that trial was approximately 50 percent, and all responses were partial responses. All patients required dose reductions because of progressive interferon-related toxic side effects. In this protocol, we attempted to administer interferon cyclically at a dose of 50 million units per meter squared intramuscularly daily for four consecutive days (following an initial dose of 10 million units/m^2 on day 1) every third week. The hope was that patients would recover from interferon-related side effects and that the amount of interferon actually delivered to patients might be increased by administering the therapy in this manner. If this was possible, higher response rates might be attained. Twenty-four patients were entered of whom 22 are evaluable for response. There was one complete remission lasting 7+ months, six partial remissions lasting a median of 7.5 months, six minor responses, and nine patients without response. In the first twelve weeks of therapy, 94.5 percent of the projected total dose of interferon had been administered reflecting reasonable patient tolerance to this different schedule of interferon administration. This compares to a less than 50 percent projected delivery of drug in patients treated with 50 million units intramuscularly three times per week on the older protocol. The similar overall response rate to the original study suggests that there is no dose-response relationship of this disease with interferon alfa at least over the dose range examined.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Dan Ihde	Senior Investigator	NMOB, NCI
John D. Minna	Chief	NMOB, NCI
Edward Sausville	Senior Investigator	NMOB, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
John W. Smith II	Senior Staff Fellow	CRB, NCI
Robin L. Miller	Senior Staff Fellow	CRB, NCI

OBJECTIVES

1. To study the clinical efficacy of a high-dose intermittent schedule of recombinant alfa interferon in patients with cutaneous T-cell lymphoma.

2. To determine the safety of this schedule of interferon administration.

METHODS EMPLOYED

Previous studies using recombinant alfa interferon in patients with relapsed cutaneous T-cell lymphoma have shown significant activity of this drug. However, the dose used was the maximal tolerate dose, 50 million units per m^2 administered intramuscularly three times per week continuously. Dose reductions were necessary in all patients entering this trial because of excessive toxicity. Although partial response occurred in approximately 50 percent of patients, they were of fairly short duration, and no complete response were observed.

The results of this study suggested that although interferon administered via this dose and schedule was active, dose limiting toxicity prevented the continuous administration of full doses. Perhaps higher response rates and better tolerance of the therapy could be obtained if the schedule of interferon administration was altered. The dose and schedule to be used in this study, therefore, was changed so that patients would receive 10 million units per m^2 intramuscularly on day 1 followed by 50 million units per m2 on days 2 through 5. This schedule of administration would then be repeated every three weeks. It was hoped that by altering the schedule of interferon administration as outlined in this protocol that more drug might be administered and dose reductions would not be required as often. The escalation of the dose to 100 million units/m^2 on days 2 through 5 in patients with at least stable disease on the lower dose after 3 cycles would test the hypothesis that there is a dose response relationship in patients with cutaneous T-cell lymphoma to interferon.

MAJOR FINDINGS

This study was a collaborative study between the Biological Response Modifiers Program and the Navy Medical Oncology Branch.

Twenty-four patients were entered on this study of whom 22 are evaluable for response. One patient was removed after a single dose of interferon because of the development of angina, and one patient was removed after three doses of interferon because of severe myalgias and headaches. There was one complete response that is ongoing at seven months and six partial responses lasting four, six, seven, eight, fourteen, and twenty-six months. There were six minor responses and nine patients without response. Seven patients underwent a dose escalation to 100 million units/m^2 without improvement in their response status. During the first twelve weeks of administration of interferon, 94.5 percent of the projected total dose of interferon had been administered and is to be compared to a less than 50 percent projected total dose delivery on the prior study using 50 million units/m^2 given intramuscularly three times per week. Of the patients on the current study, only 23 percent (of the 22 patients evaluable on the study) had any dose reduction during the first twelve weeks of treatment.

Thus, this regimen of interferon administration appears to be better tolerated, and higher total doses can be administered than on the three times per week schedule previously employed. Although there is activity with this method of interferon administration, it cannot be stated whether it is more effective or less effective than the schedule previously employed.

SIGNIFICANCE

Higher doses of interferon can be administered to patients with cutaneous T-cell lymphoma using this q 3 week schedule of drug administration. This results in some antitumor responses that appear to be comparable in degree and in percentage response rate, however, to the previously employed continuous method of interferon administration. Thus, despite delivering a greater dose intensity, response rates appear not to have significantly changed. Thus, with the patient population that was studied in this protocol and comparing this method of interferon administration to the one used previously, there does not appear to be a major dose-response relationship apparent.

PROPOSED COURSE

This study has been closed to further patient entry, and future studies with interferon alfa and deoxycoformycin are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 09298-03 CRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Adoptive Immunotherapy of Cancer with Autologous LAK Cells Plus IL-2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. L. Longo	Associate Director	OAD, NCI
Others:	K. C. Conlon	Staff Fellow	CRB, NCI
	R. G. Steis	Chief	CRB, NCI
	J. S. Beman	Nurse Specialist	CRB, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith	Senior Staff Fellow	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc., Frederick, MD (W. J. Urba)

LAB/BRANCH
Clinical Research Branch

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Exposure of peripheral blood mononuclear cells from cancer patients to high concentrations of recombinant IL-2 in vitro has been observed to result in the generation of cells capable of causing lysis of a variety of tumor cells. This study is an attempt to utilize such cells in the therapy of cancer in humans. Previous studies in the Surgery Branch of the National Cancer Institute demonstrated significant antitumor activity of these cells when administered concurrently with IL-2. On our initial trial (see last year's summary), 30 patients were treated with a bolus IL-2 regimen and 27 were evaluable for response, 2 of whom had partial responses. In order to evaluate the toxicity of a continuous infusion IL-2 plus LAK cell regimen and to determine in a preliminary fashion its antitumor efficacy, we have performed a trial using 5 days of continuous infusion IL-2 priming, 3 days of leukapheresis with LAK cells generated from each of these days, and then 5 days of continuous infusion IL-2 along with reinfusion of the LAK cells. To date, 20 patients have been treated with this regimen with 12 patients evaluable for response. Toxicities have been quantitatively similar to the bolus regimen but qualitatively significantly less severe. One of three patients with renal cell cancer has had a partial response. There have been no responses in patients with other diseases.

PROJECT DESCRIPTION

PERSONNEL

Dan L. Longo	Associate Director	OAD, NCI
Ronald G. Steis	Chief	CRB, NCI
JoAnn S. Beman	Nurse Specialist	CRB, NCI
Jeffrey W.Clark	Medical Officer	CRB, NCI
John W. Smith	Senior Staff Fellow	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI

OBJECTIVES

To confirm and extend the observations of Dr. Steven Rosenberg and his colleagues on the efficacy of systemically administered autologous lymphokine activated killer cells plus interleukin-2.

METHODS EMPLOYED

Patients considered eligible for this study are evaluated in the outpatient clinic, and an assessment is made of their ability to tolerate the therapy. If admitted to the study, patients are given 3 million units per meter squared of interleukin-2 by continuous infusion for five days. They receive no subsequent IL-2 for two further days and then undergo a leukapheresis once daily for three consecutive days. The cells obtained after each leukapheresis are sent to the laboratory where they are purified and cultured in the presence of recombinant interleukin 2. After the third leukapheresis on a Wednesday, interleukin-2 is administered at a dose of 3 million units per meter squared and is given for five days. Cells obtained from Monday are infused on Wednesday. Cells obtained from Tuesday are infused on Thursday, and cells obtained on Wednesday are infused on the following Friday. After a two week interval, patients are retreated with another complete treatment cycle as outlined above. They are then evaluated for response, and responding patients can be retreated.

MAJOR FINDINGS

Twenty patients have now been admitted to this study and there has been one partial response observed which has persisted for four months. As expected from previous trials using high dose interleukin-2 plus LAK cells, toxicity has included greater than 10 percent weight gain, hypotension requiring pressors, oliguria, rash with pruritus, edema, fever, anorexia, cardiac arrhythmias, chills, confusion, depression, diarrhea, fatigue, skin desquamation, headache, stomatitis, myalgias, nausea and vomiting, thrombocytopenia, hyperbilirubinemia, elevations in serum creatinine, and eosinophilia.

The rebound lymphocytosis, number of LAK cells generated, and number of LAK cells given back to patients is significantly greater with the continuous infusion regimen than with our previous three-day bolus priming regimen.

SIGNIFICANCE

This study demonstrates that continuous infusion IL-2 at a dose of 3 million units per meter squared can be given with tolerable toxicity and that this regimen generates significant numbers of LAK cells. The antitumor activity of this regimen (one partial response in three renal cell cancer patients treated) appears to be similar to that of other high dose IL-2 plus LAK trials which have been reported.

PROPOSED COURSE

We plan to finish treating patients on this trial by July 1, 1988 (except for patients who may require retreatment). We are currently initiating a trial which combines IL-2 plus LAK cell therapy with chemotherapy plus alfa interferon in an attempt to evaluate whether the combination of these agents will have enhanced antitumor activity, especially against malignant melanoma and renal cell cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09304-02 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Eval. of Human Anticolorectal Carcinoma MoAb in Pats. with Dissem. Colon CA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
CoPI:	M. Bookman	Senior Staff Fellow	MB, NCI
Others:	J. W. Smith	Senior Staff Fellow	CRB, NCI
	R. L. Miller	Senior Staff Fellow	CRB, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI
	C. Pinsky	Chief Medical Officer	OAD, NCI
	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS *(if any)*

NM, CC, Bethesda, MD (J. Carrasquillo, A. Keenan, S. Larsen, J. Reynolds); PRI, Frederick, MD (W. J. Urba); Bionetics Research, Inc., Rockville, MD (R. McCabe, M. Hanna, and M. Hospel); Frederick Memorial Hospital (Valarie Dailey)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies of murine origin have been administered to large numbers of patients with cancer. In general, these antibodies have been able to localize to sites of tumor but have not brought about meaningful tumor responses. The ultimate use of such antibodies will be limited by the development of human antimouse antibody responses. Such antibodies may not only cause some toxic effects but have been shown to influence the biodistribution of antibodies and the ability of the administered antibody to reach the sites of tumor. This study was undertaken to use recently developed human monoclonal antibodies to hopefully overcome the problem of antigenicity associated with murine monoclonal antibodies. We have administered either antibody 16.88 or 28A32 to 26 patients and have not noted the development of antibodies to the administered human monoclonal antibody in any patient. Three patients had pre-existing anti-28A32 antibodies, and the titer of antibody increased somewhat in one of these patients. Positive images of tumors were obtained in 20 of the 26 patients; the reason why images were not obtained in the other six patients is unknown, but in one patient in whom an image was not obtained with antibody 28A32, an endogenous antibody with reactivity similar to 28A32 was found. Presumably this antibody acted as a "blocking antibody" and prevented the administered labeled antibody from reaching the tumor target. The ability of these antibodies to localize to sites of tumor and their lack of immunogenicity in patients with cancer suggest that they might be used to deliver therapeutic doses of radionuclides to sites of tumor.

PROJECT DESCRIPTIONS

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Michael Bookman	Senior Staff Fellow	MB, NCI
John W. Smith II	Senior Staff Fellow	CRB, NCI
Robin L. Miller	Senior Staff Fellow	CRB, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Carl Pinsky	Chief Medical Officer	OAD, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

1. Determine the in vivo toxicity, pharmacokinetics, tissue localization, and potential therapeutic benefit of 16.88 and 28A32 monoclonal antibodies in patients with colorectal carcinoma.

2. To attempt radioimaging with the antibody conjugated to ^{131}I, to study the biodistribution of the antibody, to study the effects of an increase of cold antibody on its biodistribution, and finally to determine whether the antibody will have diagnostic utility when conjugated to an isotope.

METHOD EMPLOYED

Prior to referral, tumor tissue from patients previously undergoing resection of colorectal carcinoma is tested for reactivity with both monoclonal antibodies. If the tumors are reactive with one or both antibodies, patients are evaluated in the clinic for entrance into the study. If the performance status is greater than 70 percent and measurable disease is present, patients are injected on week 1 with 8 mg of the appropriate monoclonal antibody radiolabeled with 5 millicuries of ^{131}I. In week 2 the same amount of labeled antibody is administered together with "cold" antibody of increasing amounts in consecutive groups of 3 patients. In weeks 3 through 5, "cold" antibody is administered alone in an attempt to determine if the antibody can bring about an antitumor effect. Previous studies with other antibodies have shown that when excess cold antibody is administered to patients receiving labeled antibody, localization of the radiolabeled antibody to sites of tumor can be enhanced.

MAJOR FINDINGS

Eighteen patients with tumor specimens reactive with antibody 28A32 were admitted to the study. Two of these patients had skin-test reactivity to antibody 28A32 prior to their first injection of antibody and were excluded from the imaging part of the protocol. Of the 16 patients studied, 13 had images obtained of at least one area of metastatic disease. Uptake of isotope was seen in metastatic deposits in bone, liver, lung, lymph nodes, and in a soft tissue mass in the abdominal wall. The smallest tumor mass that was imaged was a 3 x 3 cm right upper quadrant abdominal wall mass. There was a great deal of heterogeneity even within single patients as regards uptake in sites of metastatic disease; lesions of comparable size in the same patient may or may not have taken up isotope to a degree detected by the scanner. Toxicity was minimal. One patient developed a localized urticarial rash after injection number one of the antibody, and no further therapy was administered. No other toxic effects that could be attributed to antibody were seen in our patients.

Monoclonal antibody 16.88 was administered to eleven patients with metastatic colon cancer one of whom could not be evaluated for scan quality because of rapidly progressive disease. Of the remaining ten patients, seven had detectable uptake of antibody in sites of metastatic disease. Metastatic deposits were observed on the antibody scan in the lung, liver, in an epigastic mass, in bone, in a pelvic mass, and in a large brain metastasis. The smallest lesion imaged was a lung metastasis measuring 2.0 x 1.9 cm on chest X ray. Again, there was a great deal of heterogeneity as regards uptake of isotope in sites of metastatic disease of even comparable size in individual patients. No toxic effect of the antibody could be discerned in any of our patients. One patient was excluded from study because of positive skin test reactivity prior to administration of the first dose of antibody.

With each antibody it did not appear that administering cold antibody with the labeled antibody prevented the development of a positive image on the scan.

Immunogenicity of these antibodies in our patients was evaluated by an ELISA assay and a latex bead agglutination assay. Five patients had antibody to 28A32 prior to administration of this antibody. No patient developed an antibody against 28A32 during the course of the study. No patient was found to have either baseline or post-study antibodies directed against 16.88.

There was no evidence for the formation of immune complexes in the serum of any of these patients, and in no patient was a tumor response observed.

SIGNIFICANCE

This study was undertaken primarily to determine if human monoclonal antibodies of the IgM subclass were capable of reaching sites of metastatic disease following intravenous administration. We also wished to determine whether these antibodies were immunogenic in man and if they brought about any toxic effects in patients. We can conclude that these antibodies can travel to sites of tumor and that they are nonimmunogenic in man. The quality of the scans, the ability of the antibody to detect tumors down to a size of 2 x 2 cm, and the heterogeneity of the uptake of antibody at sites of tumor of varying sizes are comparable to those obtained following intravenous administration of murine monoclonal antibodies. However, the human monoclonal antibodies are not immunogenic, and if one wished to use these antibodies repetitively to deliver a therapeutic dose of radiation, this process would not be prevented by the development of human antihuman antibodies. This is the first study to use human monoclonal antibodies in patients with colon cancer, and the results suggest that it is reasonable to proceed to the treatment of human colon carcinomas by the administration of these antibodies coupled to high doses of radioactive materials.

PROPOSED COURSE

This trial will shortly be closed to patient entry since we have administered doses of these antibodies close to the maximum dose (200 mg) we had outlined for this study.

PUBLICATIONS

Smith J, Bookman M, Carrasquillo J, Larson S, Reynolds J, Dailey V, Perentesis P, Urba W, McKnight J, Clark J, McCabe R, Hanna M, Haspel M, Longo D, Steis R. Evaluation of a human anti-colorectal carcinoma monoclonal antibody in patients with metastatic colorectal cancer. In: Proceedings of the American Society of Clinical Oncology. Atlanta 1987;6:(Abst. 985)250.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09305-02 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

2' Deoxycoformycin in IFN-Resistant HCL or T Gamma Lymphoproliferative Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
Others:	D. L. Longo	Associate Director	OAD, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith II	Senior Staff Fellow	CRB, NCI
	R. L. Miller	Senior Staff Fellow	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI

See next page for continuation

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (W. J. Urba); Frederick Memorial Hospital, Frederick, MD (N. Englar)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study of deoxycoformycin therapy in patients with hairy cell leukemia or T-gamma lymphoproliferative disorder was written as a salvage protocol to treat these patients if they failed to respond to low-dose alfa interferon on other ongoing CRB protocols. Deoxycoformycin is known to have significant activity in hairy cell leukemia and has been reported to produce high complete response rates in previously untreated patients and to lead to responses in patients failing prior interferon therapy. It has unproven efficacy in the T-gamma lymphoproliferative disorder. Seven patients have been admitted to this study, four with hairy cell leukemia and three with T-gamma lymphoproliferative disorder. All four patients with hairy cell leukemia were refractory to interferon alfa-2a therapy. Three of these four patients had a substantial partial response to dCF therapy and have at this moment only minimal residual hairy cell infiltrate in the bone marrow. They have normalized their peripheral blood counts, and all have been off of deoxycoformycin therapy for a minimum of six months; all remain in stable partial remission off all therapy. One patient with hairy cell leukemia died after the second dose of dCF because of profound thrombocytopenia and refractoriness to HLA-matched platelet transfusions and extensive GI bleeding. At autopsy there had been a substantial reduction in the number of hairy cells in this patient's bone marrow. Of three patients with T-gamma lymphoproliferative disorder, one patient was a complete responder, and two patients were nonresponders. The one complete responder was an elderly woman with severe granulocytopenia. We conclude that deoxycoformycin has activity in interferon-resistant hairy cell leukemia and in some patients with T-gamma lymphoproliferative disorder. Treatment is associated with acceptable toxicity but can worsen the pancytopenia associated with either of these diseases.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Dan L. Longo	Associate Director	OAD, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
John W. Smith II	Senior Staff Fellow	CRB, NCI
Robin L. Miller	Senior Staff Fellow	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Allison Martin	Staff Fellow	DEB, NCI
Stacy Nerenstone	Staff Fellow	DEB, NCI
Seth Steinberg	Acting Head	BSDM, NCI

OBJECTIVES

1. To determine the clinical effects of low doses of 2'deoxycoformycin in patients with hairy cell leukemia or T-gamma lymphoproliferative disease refractory to or intolerant of low-dose alfa interferon.

2. Determine the biochemical consequences of the administration of 2'deoxycoformycin in patients with hairy cell leukemia or T-gamma lymphoproliferative disease.

3. To determine the immunologic consequences of the administration of deoxycoformycin in patients with hairy cell leukemia or T-gamma lymphoproliferative disease.

4. To study the effects of this therapy on the serum level of a soluble form of the IL-2 receptor.

METHODS EMPLOYED

Patients with hairy cell leukemia on our ongoing trial using single-agent alfa interferon for this disease are eligible for this study if they have progressive disease during the course of interferon administration. Patients with hairy cell leukemia are also eligible for the study if the side effects to alfa interferon on this initial study are intolerable. Patients with T-gamma lymphoproliferative disorder are eligible for this study if they have not responded to three consecutive months of administration of alfa interferon.

Once patients are deemed eligible for the study, they receive 4 mg/m^2 of deoxycoformycin intravenously every week for three consecutive weeks. Patients then receive 4 mg/m^2 on alternating weeks. Patients will receive this therapy for a minimum of 12 weeks, and if disease has stabilized, continued therapy will be given for at least 6 months unless disease progression or prohibitive toxicity occurs. If a patient achieves a complete pathologically documented remission, therapy will be continued for three more months beyond the time of complete remission. If patients have a partial response to therapy, therapy may be continued indefinitely unless serial biopsies show persisting stable amounts of disease in the bone marrow. Patients who have less than a partial response will be taken off of therapy at the six month time point. Deoxycoformycin is administered after hydration with 1 liter of D5 and half normal saline. After administration of the deoxycoformycin, one further liter of fluid is given intravenously.

MAJOR FINDINGS

Four patients with hairy cell leukemia refractory to interferon alfa-2a therapy were treated on this study. All four of these patients were found to have neutralizing anti-interferon alfa-2a antibodies that developed in association with the development of clinical resistance to interferon alfa-2a. All four patients had a very rapid reduction in the peripheral blood hairy cell count and some myelosuppression induced by the deoxycoformycin. One patient died after two doses of deoxycoformycin due to persistent severe GI bleeding associated with profound thrombocytopenia which was worsened by deoxycoformycin therapy but was present prior institution of this treatment and was related to the underlying hairy cell leukemia. The other three patients completed six months of therapy with deoxycoformycin and at the time of post-therapy clinical evaluation were found to have minimal numbers of hairy cells in the bone marrow. Peripheral blood counts in all cases had normalized. These patients have been off of treatment for a minimum of six months, and all remain in a stable partial remission. There has been no progressive increase in the number of hairy cells in the bone marrow in any of these patients since discontinuation of treatment.

Among three patients with the T-gamma lymphoproliferative disorder, there has been one complete response and two patients with no response. The patient with the complete response was an elderly female with severe granulocytopenia and a history of recurrent infections. After institution of deoxycoformycin therapy, there was a progressive increase in the peripheral blood granulocyte count and a progressive clearing of T-gamma cells from the bone marrow and peripheral blood. After approximately two months of therapy the patient developed a left sided cerebral vascular accident and was removed from deoxycoformycin therapy. Bone marrow biopsies at this time showed that she had complete clearly of T-gamma cells from the bone marrow, and she was thus felt to have been a complete responder even though she did complete the planned course of therapy. Three months after discontinuation of deoxycoformycin, a repeat bone marrow showed an increase in the number of T-gamma cells in the bone marrow suggesting that she was experiencing a bone marrow relapse. However, at this point, there has been no suppression of the peripheral blood granulocyte count, and thus, we have no plans to reinstitute treatment at this time.

We have evaluated immune function in hairy cell leukemia patients following institution of deoxycoformycin therapy. Immune function during the course of interferon treatment prior to institution of deoxycoformycin therapy was essentially normal. With institution of dCF therapy, however, there was a progressive and marked decrease in the number of T lymphocytes in the peripheral blood. This was associated with marked decreases in proliferative responses in the peripheral blood mononuclear cells of these patients and inversion of the T4/T8 ratio, and a decrease in the number of CD4 positive lymphocytes to less than 200 cells per cubic millimeter. None of these patients have developed opportunistic infections, but they have not recovered immune function as of this date. We continue to evaluate these patients for the duration and clinical consequences of this immune suppression.

Toxicities of deoxycoformycin in the setting of T-gamma lymphoproliferative disorder or advanced refractory hairy cell leukemia included myelosuppression, possibly contributing to a febrile episode in one patient, GI hemorrhage secondary to severe thrombocytopenia in one patient, anorexia, nausea, and vomiting, mild skin rash, fatigue, and chills. One patient had a pulmonary embolus felt to be secondary to a central venous catheter, and one patient had a cerebral vascular accident that occurred during placement of a central venous line. Whether this was a consequence of line placement or was incidental is impossible to determine.

SIGNIFICANCE

For patients with hairy cell leukemia, recombinant interferon alfa has been a highly successful therapy. It is clear, however, that between 5 and 10 percent of patients with hairy cell leukemia do not respond to interferon alfa, and perhaps an additional 10 percent of patients will acquire resistance to interferon alfa usually in association with anti-interferon antibodies. Optimal treatment for these patients has not been defined, but it appears from small numbers of patients reported in the literature that deoxycoformycin therapy is highly effective in this setting. Our results so far confirm previously published results from the BRMP and elsewhere but also indicate that great care should be used in administering this therapy because of its propensity to worsen pre-existing cytopenias. The immune suppression induced by deoxycoformycin in patients with hairy cell leukemia was not unexpected though the severity of immune suppression was surprising.

The current treatments for patients with T-gamma lymphoproliferative disorder are unsatisfactory. Corticosteroids, cytotoxic drugs, and splenectomy have been tried with mixed and minimal success. Deoxycoformycin was used in an attempt to treat these patients because of its previously documented effects on normal T-cell function and because of its known activity in childhood acute lymphoblastic leukemia. Although we have only treated a small number of patients, it is clear that this drug has some activity in this disease. We are aware of no other studies using deoxycoformycin in this disease, and we plan to continue to evaluate the effectiveness of this therapy in this rare leukemia.

PROPOSED COURSE

This protocol is being kept open for further patient accrual. In addition to evaluating this drug for its efficacy in this disease, we intend to carefully monitor immune function in those patients receiving deoxycoformycin and determine the long-term consequences of the immunosuppression observed.

PUBLICATIONS

Steis RG, Smith J, Urba W, Clark JW, Itri, LM, Evans LM, Schoenberger C, Longo DL. Resistance to recombinant interferon alfa 2a in hairy cell leukemia

associated with neutralizing anti-interferon antibodies, N Engl J Med 1988; 318:1409-13.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09306-02 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Phase I Evaluation of Recombinant Human Granulocyte Macrophage CSF

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
Others:	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith	Senior Staff Fellow	CRB, NCI
	R. L. Miller	Senior Staff Fellow	CRB, NCI
	D. L. Longo	Associate Director	OAD, NCI
	S. P. Creekmore	Chief	BRB, NCI
	M. B. Stewart	Commissioned Officer	BRB, NCI
	K. C. Conlon	Staff Fellow	CRB, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (W. J. Urba); Frederick Memorial Hospital, Frederick, MD (J. Hersey)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We undertook a phase Ib study of recombinant human granulocyte macrophage colony stimulating factor (GM-CSF) to determine not only the ability of this agent to stimulate myelopoiesis in vivo but also for its ability to stimulate functional activity of mature hematopoietic cells. GM-CSF was given in a dose escalation manner to consecutive groups of three patients with doses ranging from 1 up to 50 micrograms per kilogram daily. Toxic effects included fatigue, bone pain, nausea, myalgias, and headaches. Occasional patients developed rash, diarrhea, and chills. At the highest dose administered, hypotension, decreased serum albumin levels, and increased serum alkaline phosphatase levels were observed. Treatments resulted in dose-related increases in white blood cell and granulocyte counts, eosinophilia, and slight increases in monocytes and lymphocyte counts. All counts return rapidly to normal following discontinuation of treatments. There was enhancement of HLA-DR expression on peripheral blood monocytes but decreased expression of Fc receptor expression on these same cells. There was increased expression of CD11B (an adhesion molecule) on the surface of the granulocytes. There was no direct enhancement of monocyte or granulocyte function as measured by tumoricidal activity or superoxide generation respectively. Thus, although this agent had significant effects on myelopoiesis and activated monocytes as determined by HLA-DR expression, the agent itself appears to be incapable of enhancing tumoricidal activity of monocytes or granulocytes. If this agent is to be used as a tumoricidal agent, it must be coupled with an agent capable of stimulating functional activity of granulocytes and monocytes.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
John W. Smith	Senior Staff Fellow	CRB, NCI
Robin L. Miller	Senior Staff Fellow	CRB, NCI
Dan L. Longo	Associate Director	OAD, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Michael B. Stewart	Commissioned Officer	BRB, NCI
Kevin C. Conlon	Staff Fellow	CRB, NCI

OBJECTIVES

1. To determine the toxic effects of recombinant human GM-CSF in patients with cancer.

2. To determine the effects of recombinant human GM-CSF on granulocyte and monocyte function in vivo.

3. To determine the in vivo effects of recombinant human GM-CSF on marrow cellularity, peripheral white blood cell counts, circulating and marrow CFU-GM, and CFU-GEMM.

4. To determine the pharmacokinetics of intravenously administered recombinant human GM-CSF.

5. To determine the antitumor effects of recombinant human GM-CSF.

METHODS EMPLOYED

Patients to be considered for this study must have a histologically confirmed diagnosis of a solid tumor for which there is no effective therapy or for which effective therapies were previously tried and failed. We selected only those patients with a Karnofsky performance status greater than or equal to 70 percent, and those with no past history of heart disease, myeloproliferative disorder or myelodysplastic syndrome. They must have adequate pulmonary, renal, and hepatic function and must not have received radiation therapy to greater than 50 percent of their axial skeleton. Patients received no therapy for four weeks prior to entry and must not have had CNS metastases.

Consecutive groups of three patients were treated on a single dose level of recombinant human GM-CSF. The dose levels used were 1, 10, 25, and 50 micrograms per kilogram. All GM-CSF doses were administered intravenously as a one to two hour infusion. One dose of recombinant human GM-CSF was given on week 1, and if no significant toxic effects occurred, daily injections of recombinant human GM-CSF were initiated on week 2, and these continued for 21 consecutive days. Serial blood specimens were obtained prior to and during treatment to assess the state of activation of peripheral blood cells, and their numbers. In addition, a bone marrow biopsy and aspirate were obtained prior to and after completion of recombinant human GM-CSF. Samples of bone marrow and peripheral blood were also sent for determination of progenitor cell numbers as reflected in CFU-GEMM and CFU-GM assays.

MAJOR FINDINGS

A total of twelve patients were entered on this study, and one patient is inevaluable for toxicity and response due to rapid disease progression after a single dose of GM-CSF. No patient stopped therapy due to toxicity. Three patients each were treated at the 1, 10, and 25 micrograms per kilogram dose levels, and two patients received 50 micrograms per kilogram. Toxic effects included mild fatigue (eight patients), bone pain (seven patients), fever (five patients), nausea and anorexia (five patients), myalgias (four patients), flushing (three patients), chills (two patients), pruritus (two patients), rash (one patient), and diarrhea (one patient). Three patients including two at the 50 microgram per kilogram dose level had transient hypotension, the lowest blood pressure reading being 80 over 50. Hypotension and bone pain responded to slowing the rate of infusion. Other toxic effects responded to symptomatic treatment. Except for hypotension, there was no clear correlation between dose and toxicity. One patient at 50 micrograms per kilogram had a vaso-vagal episode after one treatment which did not recur after subsequent doses. No consistent hematologic changes were seen at the 1 microgram per kilogram dose level. At doses equal to or greater than 10 micrograms per kilogram, there were dose-related increases in the total white blood cell count, the absolute granulocyte count, increases in band counts, and increases in eosinophil counts. Hematologic changes occurred continually throughout the course of recombinant human GM-CSF administration and were maximal at the end of treatment before rapidly returning to baseline after treatment was discontinued. No antitumor responses were seen.

Serial bone marrow biopsies in these patients revealed a dose-dependent increase in the M to E ratio, an increase in the number of eosinophils in the marrow, but no consistent changes in CFU-GMs or CFU-GEMMs. Marrow cellularity increased substantially in patients at doses greater than 10 micrograms per kilogram, and the lack of increase in stem cell numbers (expressed as numbers of colonies per 10^5 cells plated) might be a reflection of this increased cellularity. Were we able to calculate the number of stem cell colonies per hemipelvis, stem cell numbers may have increased.

Monocytes were isolated from the peripheral blood of these patients during the course of treatment. GM-CSF administration resulted in an increase in the cell-surface expression of HLA-DR on these cells but decreased expression of Fc receptor numbers. Cell-surface expression on monocytes of the adhesion molecule Mo-1 (CD11b) increased with time as patients continued on this study. There was no enhanced killing of the A375 melanoma cell line by monocytes obtained from patients receiving recombinant human GM-CSF at any dose. There was marked variability of superoxide induction in granulocytes from blood obtained from these patients prior to administration of GM-CSF. This variability made it difficult to assess changes in this parameter of granulocyte function, but no definite changes in PMA-induced superoxide production in granulocytes exposed in vivo to GM-CSF could be shown.

SIGNIFICANCE

Colony stimulating factors might be used as biological response modifying agents to bring about tumor responses directly or in combination with other BRMs. We undertook this study to determine the toxicity of recombinant human GM-CSF but also to determine if it was possible to enhance effector cell functions in the hope that we ultimately could administer these agents with monoclonal antibodies, lymphokines, or monokines to bring about tumor regressions. The results of this study show that although we can increase effector cell numbers, these cells were incapable of lysing tumor cells in vitro and in vivo. There was clearly some effect of the GM-CSF on monocytes in that HLA-DR expression and expression of CD11b were enhanced.

Thus, if GM-CSF is to be explored further as an antitumor agent, it should be given concurrently with an agent capable of activating effector cells to an active lytic state.

PROPOSED COURSE

This study is being closed to further patient entry. We plan to soon initiate a trial of recombinant human GM-CSF administered intraperitoneally for patients with disease limited to this anatomic space. From animal studies this agent appears to be capable of trafficking peripheral blood monocytes into the peritoneal cavity. Once there, these cells could be activated with interleukin-2 or gamma interferon to become cytotoxic for tumor cells.

PUBLICATIONS

Clark J, Longo D, Smith J, Urba W, Miller R, Ruscetti F, Hursey J, Steis R. Phase I trial of recombinant human granulocyte macrophage stimulating factor (rHuGM-CSF) in cancer patients (Pts). In: Proceedings of the American Society of Clinical Oncology. New Orleans 1988;7:(Abst. 622)161.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 09307-02 CRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Phase I Evaluation of rTNF Plus rIFN-Gamma in Patients with Solid Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
CoPI:	N. Rosen	Senior Investigator	MB, NCI
Others:	D. L. Longo	Associate Director	OAD, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith	Senior Staff Fellow	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI
	M. Lippman	Head	MBCS, NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc., Frederick, MD (W. J. Urba); Frederick Memorial Hospital, Frederick, MD (M. Farrell); Genentech, San Francisco, CA (Steven Sherwin)

LAB/BRANCH
Clinical Research Branch

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical trials using single agent tumor necrosis factor have been ongoing for approximately the past 2 years. In vitro studies with TNF have demonstrated that the combination of interferon-gamma with tumor necrosis factor results in significant augmentation of antitumor effects. These results have been confirmed in vivo in animal models, however, it is not known if this enhancement is due to augmentation of the immune system. Substantial enhancement of TNF-related toxicity has also been observed using this drug in combination with interferon-gamma. In this study, we intend to investigate the immunomodulatory and toxic effects of the combination of TNF and interferon-gamma.

Thirty-six patients have been treated on this study at nine different dose levels. Toxicities observed so far include fever, chills, headache, myalgia, fatigue, anorexia, nausea, vomiting, paresthesias, pain and erythema at the injection site of rTNF, moderate granulocytopenia and thrombocytopenia, moderate elevation in liver enzymes, and increases in fasting serum triglyceride levels. One patient experienced a mild cerebral infarct and was removed from the study. At dose level 9 (100 mcg/ml IFN-gamma/100 mcg/ml rTNF), one patient had grade 4 fever, and another patient had grade 3 thrombocytopenia. The dose immediately below that, dose level 8, (100 mcg/ml rIFN-gamma/50 mcg/ml rTNF) was established as the maximum tolerated dose which should be investigated further in phase II trials. Minor antitumor activity was noted in two patients. An important finding of this study was the rTNF induced blunting of the expected monocyte activation by rIFN-gamma. No synergistic immunological effects were noted. Although enhanced immunomodulating effects were not observed, synergistic antitumor effects based on other mechanisms still may be observed in future phase II trials.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Neal Rosen	Senior Investigator	MB, NCI
Dan L. Longo	Associate Director	OAD, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
John W. Smith	Senior Staff Fellow	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Marc Lippman	Head	MBCS, NCI

OBJECTIVES

1. To determine the local and systemic toxicities of the combination of rIFN-gamma and rTNF when administered intramuscularly every other day for 3 consecutive weeks.

2. To determine the maximally tolerated dose of rTNF and rIFN-gamma on this treatment schedule.

3. To determine the effects of the combination of rTNF and rIFN-gamma interferon on immunologic functions including monocyte-related hydrogen peroxide generation and monocyte expression of HLA-DR and Fc receptors. In addition, the effects of the combination of the phagocytic activity of granulocytes will be determined.

METHODS EMPLOYED

Patients with a histologically confirmed diagnosis of solid tumor that demonstrated resistance to prior treatment regimens of proven therapeutic benefit were eligible for this study. Eligibility criteria also included a Karnofsky performance status of greater than 60 percent, a life expectancy of greater than three months, an interval of at least three weeks since any prior chemotherapy, radiation therapy, hormonal therapy, immunotherapy, or major surgery (or at least six weeks after treatment with nitrosoureas or mitomycin), adequate hematologic, renal, hepatic, pulmonary and metabolic function, evaluable or measurable disease, and written informed consent. Patients were excluded if they had clinically apparent vascular disease, heart disease, known bleeding disorders, CNS metastases, known seizure disorder, lymphangitic pulmonary metastases, or prior treatment with either rIFN-gamma or rTNF. The following drugs were excluded during the study: nonsteroidal anti-inflammatory drugs, corticosteroids, aspirin (except as noted in treatment plan), and vasodilators.

Pretreatment evaluation included history and physical examination, complete blood cell count, complete chemistry profile, complete coagulation profile, antibodies to rIFN-gamma and rTNF, pulmonary function tests, ECG, UA, PA and lateral chest X rays, appropriate CT and isotopic scans, and baseline tumor measurements. Patients had a complete reevaluation within 72 hours of the end of therapy and one month after treatment.

The study employed the following trial design. Patients received intramuscular injections of both rTNF and rIFN-gamma (with the rTNF given five minutes after the rIFN-gamma) into different sites every other day for three consecutive weeks for a total of ten treatments. Each patient received the same doses of each cytokine throughout the study. Patients were sequentially assigned to different dose levels of all the possible combinations of rTNF and rIFN-gamma at doses of 10 mcg/m^2, 50 mcg/m^2, and 100 mcg/m^2. Three additional patients were treated at the maximum tolerated dose with aspirin, 650 mg po qid on the day of treatment starting one hour before therapy. Patients who exhibited at least a minor response could receive additional therapy for as long as the response continued. All patients with fever greater than 38.5 degree C received acetaminophen 650 mg every four hours as needed. For uncontrolled fever, patients were treated with cold sponge baths and a cooling blanket. Severe rigors were treated with meperidine, 10 mg I.V. as needed. All patients had three baseline immune parameters obtained and serial immune parameters before and after the patient's first and eighth doses. Hydrogen peroxide generation by monocytes, Fc receptor number, and HLA-DR expression on peripheral blood monocytes were assayed to determine the extent of monocyte activation.

MAJOR FINDINGS

Thirty-six patients were entered onto this trial. The median age was 52 years (range 23 to 72). The group consisted of 22 male and 14 female patients. Sixteen of the patients had colon cancer, six had melanoma, four had renal cell cancer, and ten had other adenocarcinomas. Fifty-eight percent had received prior chemotherapy, 19 percent prior radiation therapy, 33 percent prior immunotherapy, and only 19 percent had received no prior treatment.

All patients experienced some fatigue with treatment, and 36 percent of the patients spent more than 50 percent of their time in bed on treatment days. Ninety-seven percent of patients experienced fever, 100 percent chills, 78 percent myalgia, and 81 percent headache. Two patients had fever greater than 105 degrees Fahrenheit in spite of maximal antipyretic therapy and were removed from study. Fevers and headache were worse at the higher rTNF doses even when the rIFN-gamma doses were low. Anorexia was noted in 81 percent of the patients, but only 17 percent of the patients had significant weight loss. Thirty-six percent of the patients had nausea alone, and 28 percent had nausea and vomiting, however, none was severe. Eleven percent of the patients had mild paresthesias. All but four patients experienced pain and erythema at the injection site of rTNF, but this toxicity was not dose limiting in any patient. Skin necrosis or ulceration was not seen in any patient, and only three patients (8 percent) had induration. No patient had inflammation at rIFN-gamma sites. Thirty-six percent of patients had transient hypotension, only 11 percent had a greater than 25 percent decrease in their systolic blood pressure. Hypotension responded to I.V. fluids in all cases and was not a dose-limiting toxicity in any patient. No relationship to dose was evident.

Only five patients had decreases in their granulocyte count to less than 1,500/mm^3, and none had less than 1,000/mm^3. Decreases in WBC and granulocytes occurred only at doses of 100 mcg/m^2 rIFN-gamma and were transient (observed only once) in all cases. Twelve patients, 33 percent, actually had transient slight increases in their granulocyte count. Overall, only six patients had declines in their platelet count, while eight patients had moderate increases. Only two patients (6 percent) had decreases in their platelet count to less than 100,000/mm^3. One patient who had previously received multiple treatments with radiolabeled anti-ferritin antibodies had a platelet count nadir of 43,000/mm^3 (grade 3 toxicity) and was removed from study. Nineteen patients (53 percent) had moderate decreases in hemoglobin levels. Only two patients received blood transfusions.

Normal values for PT, PTT, TT, FDP were noted in all patients throughout the course of treatment. Several patients had baseline elevations of fibrinogen that remained unchanged throughout treatment. There was no clinical evidence for disseminated intravascular coagulation in any patient; however, one patient did experience a mild cerebral infarct and was removed from the study.

Mild to moderate increases in SGOT were noted in 44 percent of the patients, many of whom had disease metastatic to liver which progressed during the study period. No significant changes were noted in BUN or creatinine related to treatment. Forty-four percent of patients had transient microscopic hematuria; however, no abnormalities were detected in those patients who did undergo cystoscopy.

Fasting serum triglyceride levels increased in all but three patients during treatment. The average increase among the different dose levels ranged from 18 percent to 75 percent with a trend toward greater increases with higher rTNF doses. No significant changes were noted in serum cholesterol levels.

The dose level immediately below that level which produced grade 3 or grade 4 toxicity in at least two patients was defined as the maximum tolerated dose (MTD). At dose level 9, one patient had grade 4 fever and another patient had grade 3 thrombocytopenia; therefore, dose level 8 (100 mcg/m^2 rIFN-gamma/50 mcg/m^2 rTNF) was established as the MTD. After the MTD was determined, three additional patients were treated at the MTD with aspirin 650 milligrams po qid beginning one hour before treatment. No differences in toxicity were observed except for a slight reduction in headache severity.

One patient with malignant melanoma metastatic to multiple subcutaneous sites had a mixed response (one lesion completely regressed but others progressed). Another patient diagnosed with malignant mesothelioma metastatic to the pleura and peritoneum had malignant ascites that became cytologically negative although the production of ascites did not decrease. This patient received one maintenance course before his ascites again showed malignant cells.

In terms of immunological effects, a consistent pattern was seen. For a given dose of rIFN-gamma, increasing rTNF doses blunted the expected enhancement of Fc receptor number, HLA-DR expression, and H_2O_2 generation by peripheral blood monocytes. Assays of granulocytic function were not consistently reliable for technical reasons.

SIGNIFICANCE

This study demonstrates that rTNF and rIFN-gamma can be safely administered together by intramuscular injection in the outpatient setting. The toxicities observed in this study had been previously recognized in phase I trials of the individual agents. No new or synergistic toxicities were noted. The maximum tolerated dose was 100 mcg/m^2 (2 x 10^6 units/m^2) of rIFN-gamma combined with 50 mcg/m^2 (2 x 10^6 units/m^2) of rTNF. Dose-limiting toxicities included fever greater than 105 degrees Fahrenheit in two patients, and grade 3 thrombocytopenia in one patient. At the recommended phase II dose, the significant toxicities were moderate flu-like symptoms including grade 3 or greater fever in two out of nine patients.

Despite evidence for synergism between rTNF and rIFN-gamma on tumoricidal activation of macrophages in mice, this study indicates that rTNF inhibits the activation of human peripheral blood monocytes observed with rIFN-gamma administered alone to cancer patients. Although enhanced immunomodulating effects were not observed with the doses, route, and schedule of the combination of rTNF-gamma and rIFN used in this trial, it is possible that synergistic antitumor effects based on other mechanisms will be observed in future phase II/III trials.

PROPOSED COURSE

This protocol has been closed.

PUBLICATIONS

Urba WJ, Clark JW, Longo DL, Smith JW, Miller R, Kopp WC, Finch L, Gordon K, Farrell M, Creekmore S, Stewart M, Rosen N, Sherwin R, Steis RG. Phase I evaluation of the toxicity and immunomodulating effects of tumor necrosis factor and interferon gamma. In: Proceedings of the American Society of Clinical Oncology. New Orleans 1988;7:(Abst. 641)166.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09308-02 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Phase II Efficacy Study of Roferon A (RO22-8181/002) in Hairy Cell Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Steis	Chief	CRB, NCI
Others:	D. L. Longo	Associate Director	OAD, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith II	Senior Staff Fellow	CRB, NCI
	R. L. Miller	Senior Staff Fellow	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI

COOPERATING UNITS (if any)

Program Resources, Inc., Frederick, MD (W. J. Urba); Frederick Memorial Hospital, Frederick, MD (C. Schoenberger)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is an ongoing study of the efficacy and toxicity of interferon alfa-2a in patients with hairy cell leukemia. Patients responding and tolerating this therapy are being continued on interferon at a dose of 3 million units administered subcutaneously 3 times per week. So far the therapeutic activity of interferon in this disease has been confirmed. Of 53 patients evaluable for response (out of a total of 56 entered), there were 3 complete responders, 24 partial responders, 24 minor responders, and only 2 nonresponders. Minor responses in this disease are clinically meaningful responses in that peripheral blood counts have returned to essentially normal levels, but minor responders continued to have a significant marrow hairy cell infiltrate. Of the 51 responding patients, 34 continue to receive interferon at the standard dose of 3 million units given three times per week. Three patients are continuing on interferon but require more than the standard dose of interferon to maintain their response. The six were removed from study because of progressive disease while continuing to receive interferon, and eight were removed from interferon for a variety of reasons including myocardial infarction, squamous cell carcinoma of the lung, the late development of a sensory neuropathy, depression, erythrocytosis, noncompliance, impaired cognition, and flare of a rheumatic syndrome. By far and away the most common reason for removal of patients from the study has been disease progression while they continue to receive interferon. The development of interferon resistance in these patients has been correlated with the development of anti-interferon antibodies and this latter finding is the subject of a new ongoing study at the BRMP.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Dan L. Longo	Associate Director	OAD, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
John W. Smith II	Senior Staff Fellow	CRB, NCI
Robin L. Miller	Senior Staff Fellow	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI

OBJECTIVES

To evaluate the safety and efficacy of recombinant interferon alfa in patients with hairy cell leukemia.

METHODS EMPLOYED

Patients to be considered eligible for this study must have histologically documented hairy cell leukemia and one or more cytopenias. At the initiation of therapy, all patients must have a platelet count of less than 100,000 per cubic millimeter and/or a granulocyte count of less than 1,500 per cubic millimeter and/or a hemoglobin of less than 10 grams percent or transfusion dependency. After their initial clinical evaluation, patients are then given 3 million units of recombinant alfa interferon subcutaneously daily for 4 to 6 months. In responding patients, maintenance therapy is given at a dose of 3 million units subcutaneously 3 times per week. Responding patients have continued on therapy indefinitely. Patients who experience disease progression are given escalated doses of recombinant alfa interferon in an attempt to control their disease. If dose-limiting side effects develop at the higher doses, patients are taken off study.

MAJOR FINDINGS

So far, 56 patients have been entered on this trial of whom 53 are evaluable for response. Three patients were not evaluable because one was found to have a glioblastoma multiforma, one died of a Rhizopus brain abscess, and one was not evaluable for hematologic response because she had painful bony infiltration with hairy cells but with normal peripheral blood counts. Of the 53 patients evaluable for response, there were 3 complete responders, 24 partial responders, 24 minor responders, and 2 nonresponders. These responses were attained with minimal toxicity including minor fatigue, chills, myalgia, fever, anorexia, weight loss, minor and asymptomatic elevations in liver function tests, minor alopecia, and minor impotence.

A part of this study which has assumed increasing importance has been the follow-up of patients initially responding to interferon. Of the 51 patients initially responding to interferon, 34 continue to have a response to interferon at the standard dose of 3×10^6 units subcutaneously 3 times per week. Three patients are responding to interferon but are requiring more than this amount of interferon to maintain the response. Six patients were removed from interferon due to disease progression, and eight further patients were removed from interferon for a variety of reasons as mentioned previously.

Interferon resistance in this study has been associated with the development of anti-interferon antibody responses. Of 16 patients who developed neutralizing antibodies on this study, 6 developed interferon resistance; 15 patients developed only non-neutralizing antibodies, and none of these patients had clear cut interferon resistance. Importantly, of 20 patients who did not develop anti-interferon antibodies, none showed interferon resistance. The association of interferon resistance with the presence of neutralizing anti-interferon antibodies is statistically significant ($p_2 = 0.008$). Interestingly, the neutralizing anti-interferon antibodies found in this study neutralized only recombinant interferon alfa-2a; when these sera were tested for their neutralizing ability against a preparation of partially purified natural interferon, no neutralizing activity was found. Thus, if these antibodies do in fact mediate the observed interferon resistance, this resistance could in theory be overcome by the administration of partially purified natural interferon. The observation of interferon resistance associated with anti-interferon antibodies is the subject of a new BRMP protocol (Project Number Z01 CM 0909321-01). Importantly, the development of neutralizing anti-interferon antibodies was observed in all patients who developed acquired interferon resistance; we did not observe acquired interferon resistance in any patient in the absence of a neutralizing anti-interferon antibody.

Responding patients on this study continue to receive recombinant interferon alfa-2a at a dose of 3 million units three times per week. We intend to maintain these patients on this dose indefinitely as long as the interferon is tolerated and as long as therapeutic efficacy can be shown.

This study was undertaken to evaluate the long-term efficacy of interferon alfa-2a in patients with hairy cell leukemia. This drug clearly is active in this disease, but it appears to be incapable of curing the disease in the vast majority of patients. However, there is no question that even in the absence of cure these patients do well and have essentially normal peripheral blood counts. The optimal management of hairy cell leukemia patients following the attainment of a minor, partial, or complete response with interferon therapy has yet to be defined. It appears that simply discontinuing interferon will result in disease progression and progressive cytopenias in the majority of patients anywhere from three to twelve months after discontinuation of treatment. Thus, intermittent therapy with interferon, continuous maintenance therapy with interferon, or very low-dose maintenance treatments with interferon will be necessary for patients with this disease. In our study, we plan to continually evaluate these patients for the development of late, toxic effects and for the development of interferon resistance. This information will be compared with the development of these complications in patients receiving intermittent post-induction therapy with interferon and through this comparison, some estimate of long-term efficacy and optimal long-term therapy can be attained. Because it appears that the major single reason for removal of patients from interferon is because of disease progression after initial response, the development of antibodies to interferon and correlation with the clinical course of the patient will become an important parameter to follow in the future.

PROPOSED COURSE

This study has been closed to new patient entry but is being kept open to provide drug for patients continuing to receive therapy on this protocol. As mentioned, we will follow patients closely for the development of late toxic effects, the development of anti-interferon antibodies, the development of interferon resistance, and the development of other late complications of therapy to help determine whether chronic maintenance therapy with interferon is the most appropriate form of therapy to administer to these patients following an initial course of induction therapy.

PUBLICATIONS

Foon KA, Maluish AE, Abrams PG, et al. Recombinant leukocyte A interferon therapy for advanced hairy cell leukemia: therapeutic and immunologic results, Am J Med 1986; 80:351-6.

Steis RG, Smith J, Urba W, Clark JW, Itri LM, Evans LM, Schoenberger C, Longo DL. Resistance to recombinant interferon alfa 2a in hairy cell leukemia associated with neutralizing anti-interferon antibodies, N Engl J Med 1988; 318:1409-13.

Steis R, Smith J, Urba W, Clark J, Itri L, Evans L, Schoenberger C, Longo D. Resistance to recombinant interferon alfa 2a in hairy cell leukemia associated with anti-interferon antibodies. In: Proceedings of the American Society of Clinical Oncology. New Orleans 1988;7:(Abst. 620)161.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09313-01 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Phase I Study of Liposomal CGP 10835A Muramyl Tripeptide Derivative (MTP-PE)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. W. Clark	Medical Officer	CRB, NCI
Others:	R. G. Steis	Chief	CRB, NCI
	D. L. Longo	Associate Director	BRMP, NCI
	J. W. Smith	Senior Staff Fellow	CRB, NCI
	K. C. Conlon	Staff Fellow	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI

COOPERATING UNITS (if any)

Program Resources, Inc., Frederick, MD (W. J. Urba); Frederick Memorial Hospital, Frederick, MD (S. Snow)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

CGP19835A, (MTP-PE) a totally synthetic muramyl tripeptide derivative related in structure to bacterial cell wall constituents, is a biologic response modifier that is believed to act by the mechanism of macrophage activation. It has shown activity against a number of murine tumor systems. Monocytes activated in vitro by MTP-PE have been shown to have in vitro activity against human tumors as well as in vitro and in vivo activity against bacterial, viral, and fungal infections. Patients are considered eligible for this study if they have evaluable or measurable cancer other than hairy cell leukemia and have failed or have no known effective conventional therapy. Patients are given a dose of MTP-PE intravenously once weekly for eight weeks. The study is an interpatient dose escalation trial with four patients at each dose. Dose levels are 0.1, 0.2, 0.4, 0.8, 1.2, 1.8, and 2.7 mg per meter squared. In addition to evaluating for toxicity and antitumor effect, patients have studies to evaluate whether the MTP-PE is activating their macrophages. Eighteen patients have been admitted to this study. The most frequent side effects have been fevers and chills occurring while or soon after the MTP-PE is infused and rapidly resolving. Mild increases or decreases in blood pressure which are transient have also been seen. There have been no chronic or serious toxicities. No antitumor responses have been seen although several patients have had stable disease, and two of these have received an additional 8 week course of treatment. Monocyte functional studies are still being evaluated. This study demonstrates that MTP-PE can be safely given by IV bolus up to at least a dose of 1.8 mg per meter squared (our current dose level) without serious toxicity. Once the maximum tolerated dose and the dose which most effectively activates monocytes have been determined, then phase II studies can be initiated to evaluate antitumor efficacy.

PROJECT DESCRIPTION

PERSONNEL

Jeffrey W. Clark	Medical Officer	CRB, NCI
Ronald G. Steis	Chief	CRB, NCI
Dan L. Longo	Associate Director	BRMP, NCI
John W. Smith	Senior Staff Fellow	CRB, NCI
Kevin C. Conlon	Staff Fellow	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI

OBJECTIVES

1) To introduce into the clinic and to establish the safety in humans of CGP19835A encapsulated in liposomes given intravenously,

2) To evaluate macrophage (monocyte) activation produced by CGP19835A,

3) To observe any antitumor effect which CGP19835A may produce in the patients treated with the agent, and

4) To study the principal side effects of CGP19835A.

METHODS EMPLOYED

CGP19835A, (MTP-PE) a totally synthetic muramyl tripeptide derivative related in structure to bacterial cell wall constituents, is a biologic response modifier that is believed to act by the mechanism of macrophage activation. It has shown activity against a number of murine tumor systems. Monocytes activated in vitro by MTP-PE have been shown to have in vitro activity against human tumors as well as in vitro and in vivo activity against bacterial, viral, and fungal infections. Patients are considered eligible for this study if they have evaluable or measurable cancer other than hairy cell leukemia and have failed or have no known effective conventional therapy. Patients are given a dose of MTP-PE intravenously once weekly for eight weeks. The study is an interpatient dose escalation trial with four patients at each dose. Dose levels are 0.1, 0.2, 0.4, 0.8, 1.2, 1.8, and 2.7 mg per meter squared. In addition to evaluating for toxicity and antitumor effect, patients have studies to evaluate whether the MTP-PE is activating their macrophages.

MAJOR FINDINGS

Eighteen patients have been admitted to this study. The most frequent side effects have been fevers and chills occurring while or soon after the MTP-PE is infused and rapidly resolving. Mild increases or decreases in blood pressure which are transient have also been seen. There have been no chronic or serious toxicities. No antitumor responses have been seen although several patients have had stable disease, and two of these have received an additional 8 week course of treatment. Monocyte functional studies are still being evaluated.

SIGNIFICANCE

This study demonstrates that MTP-PE can be safely given by IV bolus up to at least a dose of 1.8 mg per meter squared (our current dose level) without serious toxicity. Once the maximum tolerated dose and the dose which most effectively activates monocytes have been determined, then phase II studies can be initiated to evaluate antitumor efficacy.

PROPOSED COURSE

We plan to enter seven more patients on this study which will complete it and to continue the analysis of the effects of MTP-PE on various aspects of monocyte function in order to determine the optimal immunomodulating dose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09314-01 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Autologous Activated Monocytes Given I.P. for Colonic Carcinomatosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. W. Smith II	Senior Staff Fellow	CRB, NCI
Others:	R. G. Steis	Chief	CRB, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI
	D. L. Longo	Associate Director	OAD, NCI
	P. Miller	Biologist	OAD, NCI

COOPERATING UNITS *(if any)*

Nuclear Medicine, Clinical Center, NIH, Bethesda, MD (J. Carrasquillo); Program Resources, Inc., Frederick, MD (W. J. Urba)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human monocytes that have been isolated and purified from the peripheral blood have been demonstrated to be capable of killing tumor targets in vitro following activation to cytotoxicity by interferon-gamma. The purpose of this study is to determine if autologous activated monocytes can be used as an effective form of adoptive immunotherapy for colon cancer patients with disease spread to the peritoneal cavity. This protocol is based on a previous BRMP protocol which employed a similar study design; however, the patient population had undergone laparotomy and complete surgical excision of all macroscopic tumor. The patients in that protocol tolerated leukapheresis and infusions of activated monocytes along with interferon-gamma with tolerable toxicity. This study will enroll patients who have measurable macroscopic disease to determine whether adoptively transferred activated monocytes have cytocidal activity against established tumor. Because the disease is limited to the peritoneal cavity, the infused monocytes will have adequate contact with the tumor deposits, and questions of trafficking should not complicate the analysis of their efficacy.

Although the protocol has been open to patient entry for the past year, no patients were enrolled on the protocol due to the existence of a competing protocol using intraperitoneally administered interleukin-2 plus LAK cells. Now that that protocol has been closed, it is expected that patient accrual will be significant for this study over the next two years.

PROJECT DESCRIPTION

PERSONNEL

John W. Smith II	Senior Staff Fellow	CRB, NCI
Ronald G. Steis	Chief	CRB, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Dan L. Longo	Associate Director	OAD, NCI
Paul Miller	Biologist	OAD, NCI

OBJECTIVES

1. To measure the clinical effects including antitumor response rate of intraperitoneal infusion of autologous cytotoxic monocytes activated and boosted with recombinant interferon-gamma into patients with peritoneal colorectal carcinomatosis.

2. To determine the tumoricidal activity in vitro of monocytes removed from the peritoneal cavity of patients following intraperitoneal infusion and daily in vivo boosting with interferon-gamma.

3. To monitor monocyte trafficking of cells following intraperitoneal infusion using ^{111}indium-labeled monocytes and whole body scanning.

METHODS EMPLOYED

The criteria for patient selection are:

1. Patients must have biopsy proven colorectal carcinoma with peritoneal metastases.

2. Patients must have macroscopic disease that is measurable or evaluable either on CAT scan of the abdomen or at peritoneoscopy or laparotomy. Patients with metastases outside the peritoneal cavity are not eligible.

3. Patients must have a Karnofsky performance status of greater than 70 percent, a life expectancy greater than six months, and must not have received any anti-neoplastic therapy for at least one month prior to the study.

4. Patients must have adequate hematologic, hepatic, renal, cardiac, and pulmonary function.

5. Patients may not have received therapy with interferon-gamma in the past.

Initial evaluation will consist of a history and physical, chest X ray, CAT scans of the chest, abdomen, and pelvis, urinalysis, CBC, Chem 20, EKG, CEA assay, hepatitis B surface antigen, and HIV antibody. In addition, all patients will undergo peritoneoscopy or laparotomy prior to the start of treatment at which time a Tenckhoff catheter will be inserted and multiple biopsies will be obtained.

One week after the insertion of a Tenckhoff catheter patients will undergo cytapheresis after which monocytes will be purified by countercurrent elutriation and will be cultured with recombinant gamma interferon overnight. The following day, these activated monocytes will be infused into the patient along with interferon gamma 1,000 units/ml in two liters of dialysis fluid. Patients will receive daily infusions intraperitoneally of interferon-gamma in dialysis fluid. Cytapheresis and monocyte infusions will be repeated weekly for a total of eight infusions unless disease progresses during this time. During the middle of therapy, patients will be infused with radiolabeled monocytes and imaged at the Nuclear Medicine Department at the NIH. At the end of eight weeks of therapy, the patients will have a repeat peritoneoscopy to assess response to treatment. Patients without progressive disease will receive eight more weeks of identical therapy followed by repeat evaluation. At sixteen weeks, therapy will stop for all patients who have had progressive disease or no more than stable disease. Patients with a partial response will receive identical therapy with repeat evaluations every eight weeks and continue therapy as long as they remain in partial response or for eight weeks after they achieve a complete response.

Each time the patient is leukapheresed and patient's monocytes are purified and activated, the in vitro antitumor activity of the isolated monocytes and the patient's interferon activated monocytes will be assayed against human colon cancer cell lines. In addition, cells will be harvested from the peritoneal fluid via the Tenckhoff catheter periodically, and their cytotoxic function will also be assayed in vitro.

MAJOR FINDINGS

To date, no patients have been enrolled on this protocol.

SIGNIFICANCE

Not applicable.

PROPOSED COURSE

A total enrollment of at least 14 patients is planned for this trial. If no antitumor effects are noted in these first 14 patients, the trial will be closed. If antitumor effects are noted in these first 14 patients, additional patients will be accrued onto the study to more accurately determine the true response rate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09319-01 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunomod. Effects of Recombinant Gamma IFN in Patients With Malignant Melanoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
Others:	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (A. Maluish, W. Urba, E. Crisp, K. Gordon, D. Coggin, R. Overton); Genentech, San Francisco, CA (S. Sherwin); Frederick Memorial Hospital, Frederick, MD (R. Williams)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Preclinical studies of many biological response modifying agents have indicated that higher drug doses do not necessarily correlate with greater degrees of either immune modulation or antitumor effects. The curve relating tumor responses to BRM dose is bell shaped such that optimal modulation occurs at doses usually well below the maximally tolerated dose. In this study we attempted to determine the optimal biologic response modifying dose of gamma interferon in patients with malignant melanoma with minimal tumor burden. This group of patients was chosen for this study so as to minimize the effects of cancer on the immune parameters we wished to monitor for this study. Immunological parameters followed in this study included natural killer cell activity, hydrogen peroxide production by monocytes, and changes in the expression of Fc receptors and HLA-DR on peripheral blood monocytes. Following intramuscular administration of gamma interferon, changes in all of these parameters were seen at doses above 0.01 mg/m^2 and appeared to be maximized at the 0.1 mg/m^2 dose. At a dose near the maximally tolerated dose, 0.25 mg/m^2, although hydrogen peroxide production was enhanced, it declined significantly during the last week of interferon administration. We conclude that in humans, as in animals, there is an optimal biologic response modifying dose of recombinant interferon gamma below the MTD. The antitumor activity of this optimal biologic response modifying dose should be evaluated in this patient population.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

1. To determine the optimal biologic response modifying dose for recombinant interferon gamma in patients with malignant melanoma and minimal tumor burden.

2. To assess the feasibility of the daily administration of low-dose recombinant gamma interferon in this patient population.

METHODS EMPLOYED

Patients for this study must have a histologically confirmed diagnosis of malignant melanoma that was either Clark's level 4 or 5 with negative regional lymph nodes or Clark's level 3 or deeper lesions with involvement of regional lymph nodes, or patients with regional recurrence with all detectable tumors resected. Thus, all patients in this study were clinically disease free but at risk for recurrence of malignant melanoma. All must have had a performance status in excess of 80 percent on the Karnofsky scale and must not have had any evidence of metastatic disease or other chronic illnesses. No patient had received prior interferon therapy, and all must have had essentially normal hematologic, renal, hepatic, and endocrine function. Patients did not take aspirin or nonsteroidal anti-inflammatory agents or other forms of chemotherapy, immunotherapy or hormonal therapy for their tumor. All patients after initial evaluation received a two-week course of recombinant interferon gamma administered daily by intramuscular injection. After the initial two-week course of injections, there was a three-week rest period after which a second two-week course of therapy was administered at a different dose level. The four dose levels analyzed during the first phase of the study were 0.0001 mg/m^2 daily, 0.001 mg/m^2 daily, 0.01 mg/m^2 daily, and 0.1 mg/m^2 daily. After analysis of the immunologic results obtained from patients treated at these four dose levels, an additional dose level at 0.25 mg/m^2 daily was added. Thus, five patients were treated at each of five different dose levels representing a dose range of greater than 4 logs.

After the completion of this initial phase of the study using intramuscularly administered gamma interferon, a second phase of the study was initiated using the 0.1 mg/m^2 dose. Daily subcutaneous administration was compared to three times per week administration.

MAJOR FINDINGS

Interferon gamma was well tolerated in all patients with non-dose limiting constitutional symptoms occurring in the majority of patients at and above 0.1

mg/m^2. All five patients receiving 0.25 mg/m^2, however, developed elevated transaminase levels which in one case led to discontinuation of therapy. Immunological activity was assessed serially as mentioned above. No changes in immune function were observed at the two lowest doses. At the 0.01 mg/m^2 dose level, all immune parameters were elevated but not as consistently nor to the same levels as seen following administration of 0.1 mg/m^2. At 0.25 mg/m^2 hydrogen peroxide production was enhanced by peripheral blood monocytes, but unlike at the 0.1 mg/m^2 level, hydrogen peroxide production declined during the last several days of interferon administration. Subcutaneous administration was compared with IM administration using the 0.1 mg/m^2 dose. Subcutaneous administration resulted in enhanced hydrogen peroxide production and Fc receptor repression by monocytes. More consistent elevations in peroxide generation and higher levels of Fc receptor expression were seen following subcutaneous administration. No significant difference, however, was found between the two routes of administration. A comparison of two schedules, daily and three times weekly, suggested that monocyte activation may return to normal 72 hours after interferon administration. Of the doses tested, 0.1 mg/m^2 administered daily appeared to be the most effective biological response modifying dose. Because of ease of administration, the subcutaneous route of administration of gamma interferon is the preferred route of administration if one wishes to augment immune function for prolonged periods of time.

SIGNIFICANCE

Biological response modifiers have been used for several years in an attempt to bring about regressions of cancer. The mechanism of action of many of the BRMs at the site of the tumor is unknown in many cases. However, animal studies have suggested that optimal biological response modifying doses and optimal antitumor doses of various biologics may be substantially lower than maximally tolerated doses of these drugs. If the same phenomenon is true in patients with cancer receiving biologics, the proper application of biological agents to treat cancer should require an initial assessment of the dose that brings about optimal biologic response modification. Once this dose has been found, it perhaps might be more effective at bringing about tumor regressions than the maximally tolerated dose. The major problem with this approach has been our inability to determine the optimal biologic response modifying dose with biologic agents. In the case of gamma interferon, a number of effects of this biologic on the immune system could be monitored and if the changes in these parameters were consistent from measurement to measurement, one might be able to determine an optimal biologic response modifying dose.

Now that an optimal biologic response modifying dose has been determined with this agent, the antitumor effects of the same dose should be determined in the same patient population. In addition if the optimal biologic response modifying dose of this agent in the minimal-disease setting is the same as in the advanced disease setting, then one can also apply the findings in this study for the treatment of advanced disease. As a result of this study, two additional studies have been started. The first is by the Southwest Oncology Group which will randomize patients with malignant melanoma rendered disease free by surgery but at high risk for recurrence to either observation or the optimal biologic response modifying dose of gamma interferon. The second study will be in patients with advanced malignant melanoma and will be conducted at the BRMP. In this study we hope to determine the optimal biologic response modifying dose in the setting of advanced disease and apply that dose to the treatment of measurable metastatic disease.

PROPOSED COURSE

This study has been closed to further patient entry, but the results of this study have been used in the design of two additional studies as outlined above.

PUBLICATIONS

Maluish AE, Urba WJ, Longo DL, Overton WR, Coggin D, Crisp ER, Williams R, Sherwin SA, Gordon K, Steis RG. The determination of an immunologically active dose of interferon gamma in patients with melanoma, J Clin Oncology 1988; 6,434-45.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09320-01 CRB

PERIOD COVERED
October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interferon-Alfa With or Without Indomethacin in Melanoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
Others:	M. J. Hawkins	Head	BES, CTEP, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	J. W. Smith II	Senior Staff Fellow	CRB, NCI
	R. L. Miller	Senior Staff Fellow	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI
	S. S. Ellenberg	Statistician	CTEP, NCI
	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS *(if any)*
Program Resources, Inc., Frederick, MD (W. J. Urba, A. Maluish); Frederick Memorial Hospital, Frederick, MD (M. J. Jones)

LAB/BRANCH
Clinical Research Branch

SECTION

INSTITUTE AND LOCATION
NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was a prospective randomized trial of interferon alfa given either with or without indomethacin in patients with malignant melanoma to determine what effect, if any, indomethacin might have on the toxic, immunomodulatory and therapeutic activity of interferon in this disease. Fifty-three patients were stratified according to performance status and randomized to receive interferon alfa-2b 20 million units/m^2 intravenously five days per week for four weeks followed by 10 million units/m^2 subcutaneously three times per week either with or without indomethacin 25 mg po three times a day. The overall major response rate was 13 percent (3 complete responders and 3 partial responders among 47 evaluable patients) and was equal on both arms. Indomethacin had no effect on natural killer cell activity or induction of 2'-5' oligoadenylate synthetase in peripheral blood mononuclear cells. The mean maximal temperature elevation induced by interferon was significantly reduced (from 102.1 to 100.7, $p = 0.017$) by indomethacin, but the incidence and severity of interferon-related fatigue, performance status reductions, elevations in liver function tests, myelosuppression, headache, depression, and confusion was no different on either arm of the study. Indomethacin did not reduce the frequency of interferon dose reductions for toxic side effects and did not allow the administration of higher interferon doses. We conclude that indomethacin can reduce the fever associated with interferon therapy in patients with malignant melanoma treated with interferon without a reduction in the therapeutic or immunomodulatory activity of this agent. Because fever is rarely the dose limiting toxicity of interferon, however, indomethacin is of marginal benefit to such patients receiving interferon in the doses outlined in this study.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
Michael J. Hawkins	Head	BES, CTEP, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
John W. Smith II	Senior Staff Fellow	CRB, NCI
Robin L. Miller	Senior Staff Fellow	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Susan S. Ellenberg	Statistician	CTEP, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

1. To determine if treatment with indomethacin reduces the incidence and severity of acute and chronic toxicities associated with recombinant interferon alfa.

2. To obtain preliminary data on the effect of indomethacin on the rate of interferon induced antitumor responses in patients with metastatic malignant melanoma without hepatic or CNS metastasis.

3. To determine the effect of indomethacin administration on interferon-mediated biological responses.

METHODS EMPLOYED

To be eligible for this study patients at least 18 years of age must have a histologically documented diagnosis of metastatic malignant melanoma with measurable disease. The performance status must be EGOG 0 or 1 and patients must have an expected survival of greater than 12 weeks. Patients with active peptic ulcer disease are excluded as are patients with intolerance to nonsteroidal anti-inflammatory agents, patients with New York Heart Association classification 3 or 4 heart disease, those with ventricular arrhythmias or myocardial infarctions within the preceding six months, or those with angina. In addition, patients previously treated with interferon alfa are excluded. Patients with CNS or liver metastases are not eligible and patients must have adequate renal hepatic endocrine and hematologic function. Patients may not have received nitrosoureas or mitomycin C within six weeks prior to study entry or other chemotherapeutic agents or radiation treatment within three weeks or hormonal therapy within two weeks of entry into the study.

After initial evaluation, patients are stratified by performance status and by prior treatment with chemotherapy to receive interferon alfa either alone or with indomethacin at a dose of 20 mg po tid. The interferon dose was the same in each arm and consisted of an induction phase during which time patients received 20 million units/m^2 intravenously by slow bolus for five consecutive days each week for four weeks. Patients then received maintenance therapy at a dose of 10 million units/m^2 subcutaneously three times per week. Patients whose disease stabilized or improved after eight weeks were continued until progressive disease developed. Acetaminophen at a dose of 650 mg po was given for the control of fever associated with interferon in those patients not randomizing to receive indomethacin.

MAJOR FINDINGS

The overall major response rate was 13 percent with three complete responders and three partial responders among the 47 patients evaluable for response. The response rate was equal on both arms of the study, and thus indomethacin had no obvious effect on improving or decreasing the response rate of these patients with malignant melanoma to interferon alfa-2b. In addition, indomethacin had no effect on natural killer cell activity or induction of 2'-5' oligoadenylate synthetase activity in these patients. Interestingly, there was a statistically significant decrease in baseline NK activity in these patients with malignant melanoma compared to normal donor controls. With interferon therapy, natural killer cell activity increased to levels slightly higher than but not statistically significantly higher than the levels found in normal controls. Thus, in this study interferon whether or not given with indomethacin, acted as an immunorestorative agent at least as assessed by measurements of natural killer cell activity rather than as an enhancer of immune function.

The only statistically significant difference between the two arms of the study was that the mean maximal temperature elevation induced by interferon was reduced from 102.1 to 100.7 ($p = 0.017$) when indomethacin was administered. However, no other toxic effects of interferon were reduced by concurrent administration of indomethacin, and thus there was no difference in the frequency of interferon dose reductions for toxic effects. Higher doses of interferon could not be administered when fever was controlled with indomethacin.

SIGNIFICANCE

A large number of studies have suggested that immune function in patients with cancer might be suppressed. It has been suggested that impairment of T-cell proliferative responses in patients with cancers might be due to secretion of prostaglandins by monocytes. In addition, the proliferative responses of mononuclear cells from patients with cancer can be restored to normal by the addition of indomethacin or by depletion of monocytes. Among patients treated with interferon, there is a progressive decrease in proliferative responses of their peripheral blood mononuclear cells in vitro in response to various lectins. This suppression can be removed by the addition of indomethacin. Thus, the available data would suggest that indomethacin might be useful not only to control the fevers associated with interferon administration but might also act directly as a antitumor agent in patients with malignant melanoma. In our study, however, we were unable to find any evidence for an enhancement of the antitumor effect of interferon, and no significant effect upon the toxic effects induced by interferon save for a slight decrease in the mean maximal temperature elevation these patients had during induction therapy. Importantly, indomethacin administration did not allow for the administration of larger amounts of interferon for longer periods of time than when indomethacin was not administered. Thus, this was a negative study in terms of enhancement of immune function or of tumor responses in patients with melanoma by addition of indomethacin to interferon alfa, but it did show that one can administer interferon with cycloxygenase inhibitors to reduce fevers associated with interferon administration without concern for inhibition of antitumor effects.

PROPOSED COURSE

This study has been closed to further patient entry but is being kept open to provide drug for the few remaining patients receiving treatment on this study and to follow-up those complete responders on the study now off therapy.

PUBLICATIONS

McKnight J, Clark J, Miller R, Jones M, Smith J, Stevenson H, Urba W, Hawkins M, Longo D, Steis R. Randomized trial of recombinant interferon alpha (rIF) with or without indomethocin (IND) in patients (pts) with metastatic malignant melanoma. In: Proceedings of the American Society of Clinical Oncology. Atlanta 1987;6:(Abst. 983)250.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09321-01 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Anti-Interferon Antibodies in Hairy Cell Leukemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Steis	Chief	CRB, NCI
Others:	J. W. Smith II	Senior Staff Fellow	CRB, NCI
	K. C. Conlon	Staff Fellow	CRB, NCI
	J. W. Clark	Medical Officer	CRB, NCI
	S. P. Creekmore	Chief	BRB, NCI
	C. M. Pinsky	Chief, Medical Officer	BRB, NCI

See next page for continuation

COOPERATING UNITS *(if any)*

Program Resources, Inc., Frederick, MD (W. J. Urba); Frederick Memorial Hospital, Frederick, MD (R. Barney)

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have observed the development of interferon resistance in a number of patients receiving interferon alfa-2a treatments and have correlated it with the development of anti-interferon antibodies. Of 16 patients with neutralizing antibodies to interferon alfa-2a, 6 developed clinical resistance to this therapy. The reason why the remaining 10 patients remained sensitive to interferon in the face of antibodies is not clear, and it is the purpose of this trial to look at effects that these antibodies might have on the biological effects of interferon alfa-2a in patients with hairy cell leukemia. All patients entered on this study have hairy cell leukemia and will have been treated with interferon in the past. Patients will receive a single dose of interferon alfa-2a and serial assessments of 2'-5'oligoadenylate synthetase activity in peripheral blood mononuclear cells, serum neopterin levels, interferon pharmacokinetics, and the fever response to interferon will be made. It is anticipated that patients with neutralizing antibodies will have some inhibition of all of these biological effects of interferon alfa-2a. Because all neutralizing antibodies detected in our patients neutralized only interferon alfa-2a and not a partially purified preparation of natural interferon, these patients will subsequently receive a dose of human lymphoblastoid interferon and these same biological end points determined. If, as is anticipated, patients do have normal biological responsiveness to human lymphoblastoid interferon in the face of antibodies to interferon alfa-2a, it will be concluded that hairy cell leukemia patients with disease resistance to recombinant interferon alfa-2a will likely be responsive to therapy with human lymphoblastoid interferon.

PROJECT DESCRIPTION

PERSONNEL

Ronald G. Steis	Chief	CRB, NCI
John W. Smith II	Senior Staff Fellow	CRB, NCI
Kevin C. Conlon	Staff Fellow	CRB, NCI
Jeffrey W. Clark	Medical Officer	CRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Carl M. Pinsky	Chief, Medical Officer	BRB, NCI
Seth Steinberg	Acting Head	CDMS, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

1. To determine the effects of anti-interferon antibodies on interferon pharmacokinetics, induction of 2'-5' oligoadenylate synthetase activity, neopterin levels, and interferon-induced fevers following injection of recombinant interferon alfa-2a and human lymphoblastoid interferon.

METHODS EMPLOYED

Patients to be considered eligible for this study must have a histologically confirmed diagnosis of hairy cell leukemia and been treated for this disease with recombinant interferon alfa-2a. Prior to entry on the study patients will be evaluated for their hematologic status (CBC, differential cell count, and enumeration of TRAP positive cells in the peripheral blood), have a 24-hour urine collected for protein and creatinine clearance determinations, a serum chemistry profile, and a careful physical examination with particular attention to any history of potential allergy or other atypical reactions to interferon alfa-2a.

Patients will discontinue interferon alfa-2a (if they were receiving it prior to entering this study) at least two weeks prior to evaluation. Baseline blood samples will be obtained over three consecutive days for evaluation of anti-interferon antibody titers and in vivo levels of 2'-5' oligoadenylate synthetase activity and neopterin. Following the baseline evaluations, the patients will receive 9 million units of recombinant interferon alfa-2a subcutaneously and serial blood samples will be obtained to measure 2'-5' oligoadenylate synthetase activity, neopterin levels, and serum levels of interferon alfa-2a. After a minimum of a 48-hour washout period, patients will have the same baseline determinations of 2'-5' oligoadenylate synthetase activity and neopterin levels as mentioned previously and then will be given 9 million units of human lymphoblastoid interferon subcutaneously. Blood samples for assessment of 2'-5' oligoadenylate synthetase activity, neopterin levels, and interferon levels will be obtained as mentioned previously. In addition, following the dose of interferon alfa-2a and of human lymphoblastoid interferon, serial vital signs will be obtained to determine the maximal febrile response to each type of interferon.

After completing the evaluation of this study, patients will have completed this study and returned to their previous therapy. One month later they will return to clinic and serum will be obtained for determination of anti-interferon antibody status once again.

MAJOR FINDINGS

This study has just opened, and so far 28 patients have been evaluated to date. The blood samples have been obtained and are stored for analysis once all patients have completed their evaluation on this study.

SIGNIFICANCE

This study was undertaken to further define the clinical and biological significance of anti-interferon antibodies in patients receiving this therapy. At least in some patients, neutralizing anti-interferon antibodies appear to inhibit the effectiveness of interferon in hairy cell leukemia. The reason or reasons why all patients with neutralizing anti-interferon antibodies do not fail therapy is not clear. Perhaps these patients attained a response and are maintaining response in the face of antibodies which neutralize all of the interferon injected subsequently. Such an explanation would be in keeping with the duration of responses observed in previous studies in which interferon is discontinued in patients following an initial response to this therapy. In this case, one would still expect to find some inhibition of the biological effects of interferon. The findings in this study will have relevance to other biological response modifiers as most such agents are low molecular weight polypeptides that are potentially immunogenic. In addition, if biological responsiveness to interferon can be recovered by the use of a different and immunologically distinct preparation of interferon, those patients with hairy cell leukemia resistant to interferon because of neutralizing anti-interferon antibodies might be salvaged with an alternative interferon preparation. If biological responsiveness (for example enhancement of 2'-5' oligoadenylate synthetase and increases in neopterin levels) can be achieved with human lymphoblastoid interferon but not with interferon alfa-2a, we would conclude that treatments with this alternative interferon preparation might be efficacious in those patients resistant to interferon alfa-2a in the presence of antibodies.

PROPOSED COURSE

We will continue to accrue patients to this study and perform the necessary laboratory evaluations once all blood samples have been obtained.

PUBLICATIONS

Steis RG, Smith J, Urba W, Clark JW, Itri LM, Evans LM, Schoenberger C, Longo DL. Resistance to recombinant interferon alfa 2a in hairy cell leukemia associated with neutralizing anti-interferon antibodies, N Engl J Med 1988; 318:1409-13.

Steis R, Smith J, Urba W, Clark J, Itri L, Evans L, Schoenberger C, Longo D. Resistance to recombinant interferon alfa 2a in hairy cell leukemia associated with anti-interferon antibodies. In: Proceedings of the American Society of Clinical Oncology. New Orleans 1988;7:(Abst. 620)161.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09324-01 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Antitumor and Immunological Correlates of Individualized IL-2 Administration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. P. Creekmore	Chief	BRB, NCI
Others:	J. W. Clark	BRMP Staff Physician	CRB, NCI
	J. W. Smith II	BRMP Staff Physician	CRB, NCI
	R. G. Steis	Chief	CRB, NCI
	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS (if any)

Frederick Memorial Hospital (N. Englar); Program Resources, Inc, Frederick, Maryland 21701 (W. J. Urba, S. Markus).

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER.
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This trial was designed to investigate the anti-tumor and immunological effects of chronic IL-2 administration. In previous BRMP-sponsored studies, it has been demonstrated that endogenous LAK activity and anti-tumor responses may be produced by IL-2 administration alone, employing the treatment schedule and dosages used in this trial, without the severe toxicity and complicated ex vivo laboratory manipulations of LAK/IL-2 regimens. These prior studies have shown that in some patients, chronic outpatient administration of IL-2 alone can result in a sustained level of circulating LAK cells. In this investigation, IL-2 is given by 24-hour continuous infusion twice weekly for up to 4 weeks, at a dose of 3×10^7 U/m^2 (BRMP units) per 24 hour infusion. Subsequent IL-2 doses are adjusted in the individual patient to achieve a sustained circulating level of Leu-19 positive cells. At the end of 3 months of treatment, tumor response is assessed. No tumor responses have been seen. Patients have achieved maximum Leu-19 levels in the range of 50% of circulating mononuclear cells and elevations of white blood counts ranging above 50,000/cu mm. Circulating levels of LAK activity have been demonstrated. LAK activity and Leu-19 levels have been adequately sustained in most patients in the outpatient phase of treatment, suggesting that combination treatments may be designed around this IL-2 regimen, involving other BRMs, chemotherapy, monoclonal antibodies, etc., which might exploit or enhance the sustained levels of circulating cytotoxic cells for greater anti-tumor response. The current trial will be closed to further entry of patients after 15 evaluable patients have been treated, and is being redesigned to include moderate dose of cyclophosphamide to reduce suppressor cell populations.

PROJECT DESCRIPTION

PERSONNEL

Stephen Creekmore	Chief	BRB, NCI
Jeffrey Clark	Senior Staff Fellow	CRB, NCI
J. W. Smith II	BRMP Staff Physician	CRB, NCI
Ronald Steis	Chief	CRB, NCI
Dan L. Longo	Assoc. Director	BRMP

OBJECTIVES

The objectives of this study are:

1. To generate a maximum, sustained level of circulating LAK cells in patients using an individualize twice-weekly IL-2 dosage, which can be administered largely as an outpatient regimen.

2. To determine the long-term immune modulatory effects of IL-2 in such a regimen.

3. To determine the anti-tumor effects of such a regimen.

4. To determine the toxicity of such a regimen.

5. To investigate mechanisms of anti-tumor effects by serial tumor sampling in selected patients.

METHODS EMPLOYED

Patients are required to have measurable tumor, good performance status (70% Karnofsky or greater), expected survival greater than 3 months, and no brain metastases. Tumor types are restricted to renal cell carcinoma or malignant melanoma, with selected other tumor cell types at the discretion of the principal investigator.

Patients are treated with recombinant IL-2 (Hoffmann-LaRoche) in the following regimen:

> 3-4 weeks high-dose induction: IL-2 at 3×10^7 U/m^2 by 24 hour iv infusion b.i.w. (inpatient). Target Leu-19 level: 2500/cu mm.
>
> Chronic phase treatment: IL-2 at 3×10^6 U/m^2 by 24 hour iv infusion b.i.w. (outpatient).
>
> Dosage of chronic phase IL-2 adjusted to maintain Leu-19 levels above 1000/cu mm or 20% of maximum level attained during induction (whichever is higher). Doses >= 10^7 U/m^2 are given as inpatient, and patients may receive up to 4/8 treatments per month as inpatient.

Patients assessed for response after 3 months.

Stable or responding patients continue on therapy up to 1 year.

MAJOR FINDINGS

Fourteen patients have been entered on trial. 9 are evaluable for tumor response. No partial or complete responses have been seen, although 4 patients have had minor responses failing to meet criteria of partial response at 3 months. 2 patients were taken off study early for tumor progression, and 4 patients required dose reduction for toxicity (2 due to elevated bilirubin and 2 due to elevated serum creatinine). No patients were removed from study due to toxicity. Other significant toxicity included 3 systemic infections, hypotension requiring fluid bolus and occasional use of pressors at the higher inpatient dose levels, and other usual IL-2 toxicity. 6 patients are currently on study, 1 on the chronic treatment phase.

Patients have achieved boosts in Leu-19 levels and circulating LAK activity which have been sustained within the target range in most patients in the outpatient phase of treatment.

SIGNIFICANCE

This trial has demonstrated that the IL-2 regimen described can generate and sustain large numbers of circulating Leu-19+ mononuclear peripheral blood cells. Although there are no clinical responses in this trial so far, biologic activity is suggested by transient responses in 4 patients, and there have been documented partial responses in the previous BRMP-sponsored trial with a similar regimen (2/6 PRs in melanoma).

These results indicate that combination treatments may be designed around this IL-2 regimen, involving other BRMs, chemotherapy, monoclonal antibodies, etc., which might exploit or enhance the sustained levels of circulating cytotoxic cells for greater anti-tumor response.

Such combinations will therefore be explored in modifications of this regimen.

PROPOSED COURSE

The current trial will be closed to further entry of patients after 15 evaluable patients have been treated, and will be reopened after IRB approval to include moderate doses of cyclophosphamide to reduce suppressor cell populations. Related trials are also beginning using the same IL-2 regimen in combination with other agents (flavone acetic acid and Poly-ICLC), and a follow-on trial is written for IL-2 in the same regimen plus an ADCC-mediating monoclonal antibody, to begin when the antibody is available approximately 9/88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09325-01 CRB

PERIOD COVERED

October 1, 1987 to September 30, 1988

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Phase I Study of Flavone-8 Acetic Acid in Combination with Interleukin-2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. P. Creekmore	Chief	BRB, NCI
Others:	R. H. Wiltrout	Senior Investigator	LEI, NCI
	I. Green	Senior Investigator	BRB, NCI
	J. W. Clark	BRMP Staff Physician	CRB, NCI
	J. W. Smith II	BRMP Staff Physician	CRB, NCI
	R. G. Steis	Chief	CRB, NCI
	D. L. Longo	Associate Director	OAD, NCI

COOPERATING UNITS *(if any)*

Frederick Memorial Hospital (K. Madera); Program Resources, Inc., Frederick, Maryland 21701 (W. J. Urba, S. Markus, R. Hornung); Cancer Therapy Evaluation Program, NCI (J. L. Grem).

LAB/BRANCH

Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NCI-FCRF, Frederick, Maryland 21701

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This trial is designed to evaluate the combination of Flavone-8 acetic acid in combination with Interleukin-2. FAA as a single agent is curative for early and advanced murine colon 38, and has activity in several other murine solid tumor models. Interestingly, FAA has only modest direct cytotoxicity. In a murine renal cell carcinoma model, the combination FAA + IL-2 is curative when neither FAA nor IL-2 +/- LAK is curative at the same doses. Immunological studies in mice and men show that FAA boosts NK cell activity and induces interferons. FAA has been studied as a single agent in phase I clinical trials, without evidence of clinical activity.

The present trial is designed to explore the toxicity and immunomodulatory effects of the combination of FAA and IL-2 as a treatment for human cancer. Patients will also be monitored for anti-tumor effects of the regimen.

The trial is too early for definitive conclusions to be drawn at this time.

PROJECT DESCRIPTION

PERSONNEL

Dan L. Longo	Associate Director	OAD, NCI
Ira Green	Senior Investigator	BRB, NCI
Stephen P. Creekmore	Chief	BRB, NCI
Jeffrey W. Clark	Senior Investigator	CRB, NCI
John W. Smith II	Senior Investigator	CRB, NCI
Ronald G. Steis	Chief	CRB, NCI
Dan L. Longo	Associate Director	OAD, NCI

OBJECTIVES

The objectives of this study are:

1. To explore the immunological effects and toxicity of FAA alone and in combination with IL-2.

2. To determine optimal dosage of FAA in combination with IL-2 for immune modulation, as measured by circulating immune effector cells, and where possible determinations of lymph node and tumor infiltrating lymphocyte populations.

3. To record the anti-tumor effects of the regimen in a preliminary patient sample.

4. To evaluate anti-tumor effects and mechanisms by serial tumor sampling in selected patients.

METHODS EMPLOYED

Patients are required to have metastatic cancer without an alternative therapy of known benefit, a life expectancy of at least 4 months, measurable or evaluable disease, and adequate renal, hepatic, hematologic, cardiac, and pulmonary physiological function.

Patients are treated in 8 levels of 3 patients each. FAA (Lipha Pharmaceuticals) is given once weekly by 6-hour intravenous infusion, at doses of 8.5 gm/m^2 or 10 g/m^2. IL-2 (Hoffmann-LaRoche) doses are selected from the following levels: 1×10^6, 3×10^6, 1×10^7, or 3×10^7 U/m^2, given by 24-hour intravenous infusion.

Each patient first receives FAA as a single agent given weekly for two weeks. IL-2 is then introduced as a single weekly infusion starting 24 hours after the weekly dose of FAA, for two weeks. Dose intensity is then increased by giving IL-2 twice weekly.

Patients are monitored for toxicity and immunological changes following treatment with FAA alone and FAA with IL-2 once and twice weekly. Immune monitoring includes NK and LAK cell assays, as well as cell surface marker phenotyping. Patients are evaluable for clinical response after completing two weeks of treatment with FAA weekly plus IL-2 twice weekly.

MAJOR FINDINGS

Six patients have been entered on study. 3 have been taken off study before completion of the trial, due to tumor progression (2 with brain metastases and 1 with an intrathoracic bleed in no temporal relationship with the FAA). An additional 3 patients are now on study at the lowest dose levels of IL-2 and FAA.

Toxicity seen on the first six patients at 8.5 gm/m^2 FAA alone include grade 1-2 myalgia and weakness, increased CPK and LDH, increased template bleeding time, fatigue, diarrhea, fever, nausea, and anorexia. Grade 3 hypotension was seen in 1 patient. All toxicities resolved within 24 hours after the infusion.

No additional toxicities were noted in 3 patients receiving FAA at 8.5 g/m^2 weekly with IL-2 at 10^6 U/m^2 once weekly.

Only 1 patient so far has been treated with IL-2 twice weekly, and no additional toxicity has been introduced by the combination.

Results of immunological monitoring studies are still pending.

No patient has been treated long enough to be evaluable for clinical response, and no clinical responses have been incidentally observed.

SIGNIFICANCE

Interpretation of the trial results will have to wait until more patients have been entered.

PROPOSED COURSE

The protocol will be continued.

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